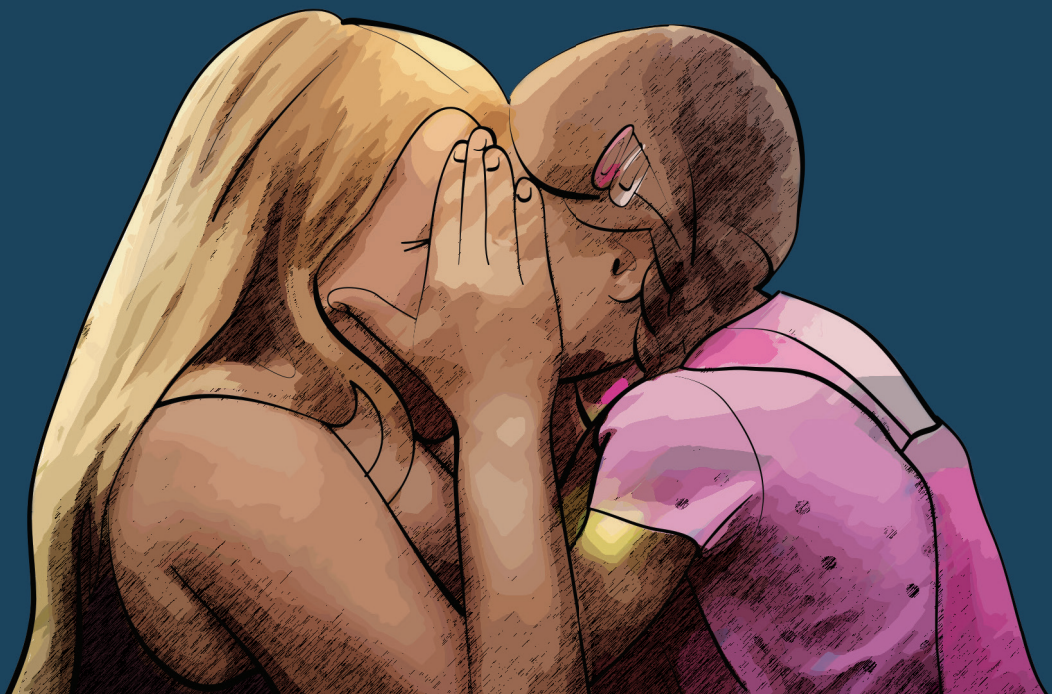
ANITA HARREWIJN

Shy parent, shy child?

DELINEATING PSYCHOPHYSIOLOGICAL ENDOPHENOTYPES OF SOCIAL ANXIETY DISORDER



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Anita Harrewijn

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Contents

Chapter 1

General introduction

One of the participants in the Leiden Family Lab study on social anxiety disorder told me she does everyday things, such as going to the hairdresser, in the next village, instead of in her hometown. She does this because she does not know how to react when she would accidently encounter an acquaintance: Should she go over and say hi? Should she just walk by? She really does not know how to behave in this social situation, and thus avoids the situation altogether.

Of course, everybody feels socially anxious or shy from time to time. Rapee and Spence (2004) propose that social anxiety can be seen as a severity continuum with on the one end people who show no anxiety at all in social situations, and on the other end patients with social anxiety disorder (SAD). SAD is an invalidating psychiatric disorder characterized by extreme fear and avoidance of one or more social situations (APA, 2013). It is diagnosed when this fear persists for more than six months, social situations are avoided or endured with intense fear, and it causes clinically significant distress or impairment in important areas of daily functioning (APA, 2013). Life-time prevalence of SAD is estimated between 5 and 13% in Western societies (De Graaf, Ten Have, Van Gool, & Van Dorsselaer, 2012; Furmark, 2002; Grant et al., 2005; Kessler, Berglund, Demler, Jin, & Walters, 2005; Rapee & Spence, 2004). SAD often co-occurs with other psychiatric disorders, such as other anxiety disorders, depression, and substance abuse (Grant et al., 2005; Rapee & Spence, 2004; Spence & Rapee, 2016). In addition to severe personal, relational, professional, and economic consequences (Acarturk, De Graaf, Van Straten, Ten Have, & Cuijpers, 2008; Dingemans, Van Vliet, Couvee, & Westenberg, 2001; Lampe, Slade, Issakidis, & Andrews, 2003; Wittchen, Stein, & Kessler, 1999), SAD is difficult to treat. For example, cognitive-behavioral therapy is less effective for SAD than for other anxiety disorders, both in children and adults (Hudson, Keers, et al., 2015; Hudson, Rapee, et al., 2015; Norton & Price, 2007; Spence & Rapee, 2016). Strikingly, the mean delay between onset of SAD and seeking treatment ranges from 14 to 28 years (Dingemans et al., 2001; Green, Hunt, & Stain, 2012; Iza et al., 2013). Thus, it is important to gain more insight in the underlying mechanisms of SAD, as this might be used to improve early detection and intervention.

Patients with SAD show information processing biases, such as biases in attention (e.g., hypervigilance, or self-focused attention), interpretation (e.g., evaluating own behavior very critically, or interpreting social situations in a negative way), memory (e.g., selectively retrieving negative information), and imagery (e.g., experiencing images of oneself performing poorly in social situations) (Bögels & Mansell, 2004; Clark & McManus, 2002;

Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013; Wong & Rapee, 2016). Haller, Kadosh, and Lau (2014) suggest that normative brain development could magnify these information processing biases in adolescents and thereby putting them at increased risk for developing SAD. Indeed, SAD usually develops in late childhood or early adolescence (Kessler et al., 2005). Moreover, these information processing biases might accumulate over time, resulting in a persistent cycle. For example, these information processing biases are triggered when the person is confronted with a socially stressful situation, repeated while in the situation, and carried forward in time when anticipating similar future events (Clark & McManus, 2002; Morrison & Heimberg, 2013). These information processing biases play an important role in the development and maintenance of SAD (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013; Wong & Rapee, 2016).

One way to study these information processing biases is by using psychophysiological measures, which provide real-time, objective and direct information with high temporal resolution (Amodio, Bartholow, & Ito, 2014; M. X. Cohen, 2011; Ibanez et al., 2012; Luck, 2005). Psychophysiological measures could be measured before, during and after social situations, and even during resting state. For example, recent studies have focused on frontal alpha asymmetry, delta-beta cross-frequency correlation (further referred to as 'delta-beta correlation'), and heart rate variability during resting state, anticipation of and recovery from stressful social situations (Chalmers, Quintana, Abbott, & Kemp, 2014; Garcia-Rubio, Espin, Hidalgo, Salvador, & Gomez-Amor, 2017; Gerlach, Wilhelm, & Roth, 2003; Grossman, Wilhelm, Kawachi, & Sparrow, 2001; Miskovic & Schmidt, 2012). Most studies on information processing biases during processing of social stimuli have focused on event-related potentials, as they provide the opportunity to differentiate between early and late processing stages (Schulz, Mothes-Lasch, & Straube, 2013; Staugaard, 2010). Faces are often used as social stimuli, but recent studies have also used social evaluative feedback as social stimulus to elicit information processing biases (Cao, Gu, Bi, Zhu, & Wu, 2015; Van der Molen et al., 2014). Recently, studies on processing social evaluative feedback in healthy participants have started to investigate neural oscillatory power. It is suggested that this might give additional information on neural activity that is not phase-locked to social evaluative feedback (Makeig, Debener, Onton, & Delorme, 2004; Van der Molen, Dekkers, Westenberg, Van der Veen, & Van der Molen, 2017). However, this has not been studied in SAD to date.

Taken together, in this dissertation I will focus on psychophysiological measures of information processing biases, to gain more insight in the mechanisms underlying the

development and maintenance of SAD. More specifically, I will focus on frontal alpha asymmetry, delta-beta correlation, and heart rate variability during resting state, anticipation of and recovery from a stressful social situation, and on the N1, feedback-related negativity (FRN), and P3 event-related potentials and theta power in response to social evaluative feedback. The goal of this dissertation is to investigate whether these psychophysiological measures are endophenotypes of SAD.

Endophenotypes

A promising line of research in psychiatry has focused on delineating endophenotypes (Glahn, Thompson, & Blangero, 2007; Gottesman & Gould, 2003). Endophenotypes are heritable trait markers 'in between' the genotype and the phenotype. Studying endophenotypes could be seen as a first step in unraveling genetic mechanisms underlying psychiatric disorders. That is, psychiatric disorders are caused by a complex interplay between many different genes. Endophenotypes are more specific measures related to the psychiatric disorder, that are supposedly related to fewer genes than these complex psychiatric disorders (Cannon & Keller, 2006; Glahn et al., 2007). Furthermore, endophenotypes could yield a better understanding of the biological mechanisms underlying SAD (Glahn et al., 2007; Iacono, Malone, & Vrieze, 2016; Miller & Rockstroh, 2013), which in turn could help in interpreting genetic findings (Flint, Timpson, & Munafo, 2014). Endophenotypes could be behavioral measures (e.g. task performance, reaction time, or questionnaire data), neural measures (e.g. (f)MRI), or psychophysiological measures (e.g. event-related potentials, neural oscillatory power, heart rate, or heart rate variability) (Gottesman & Gould, 2003). Neural and psychophysiological endophenotypes are presumed to be more closely related to the genotype than behavioral endophenotypes (Cannon & Keller, 2006). Possible endophenotypes of depression, bipolar disorder, or schizophrenia have already been investigated (Bora, Yucel, & Pantelis, 2009; Bramon et al., 2005; Dubin et al., 2012; Glahn et al., 2007; Goldstein & Klein, 2014; Gottesman & Gould, 2003). However, to date no studies have investigated putative endophenotypes of SAD. This is remarkable, given the relatively high heritability of SAD (Distel et al., 2008; Isomura et al., 2015; Kendler, Neale, Kessler, Heath, & Eaves, 1992; Middeldorp et al., 2005; Nelson et al., 2000) and the relatively high life-time prevalence (De Graaf et al., 2012; Furmark, 2002; Grant et al., 2005; Kessler et al., 2005; Rapee & Spence, 2004). Therefore, the goal of this dissertation is to delineate psychophysiological endophenotypes of SAD.

A psychophysiological measure should meet the following criteria to be seen as an endophenotype (Glahn et al., 2007; Gottesman & Gould, 2003):

1) Association with the disorder
2) Co-segregation with the disorder within families
3) Heritability
4) The endophenotypes should be seen in non-affected family members to a higher degree than in the general population
5) State-independence

The association between psychophysiological measures and SAD (first criterion) has already been studied extensively by comparing participants with and without SAD, or high and low socially anxious individuals (Miskovic & Schmidt, 2012; Schulz et al., 2013; Staugaard, 2010). The *second chapter* of this dissertation gives an overview of the most frequently studied EEG measures in social anxiety. The second and third criteria for endophenotypes are based on the observation that psychiatric disorders run in families (Glahn et al., 2007; Gottesman & Gould, 2003). Within these families, the endophenotype should be seen in persons with the disorder. Furthermore, the endophenotype should be heritable. These two criteria could best be studied in extended families instead of in twins or sibling-pairs, because of the many different types of relationships within one family. This increases the power to identify genetic variability and thereby heritability (Gur et al., 2007; Williams & Blangero, 1999). In addition, these families should be selected on two persons with the psychiatric disorder (parent and child), to ensure a focus on a genetic form and to increase the chance that endophenotypes are related to the genetic factors underlying the psychiatric disorder (Fears et al., 2014; Glahn et al., 2010). The *fourth, fifth,* and *sixth chapter* describe the results of the two-generation family study that we conducted to investigate these two criteria for endophenotypes (co-segregation and heritability). The fourth (non-affected versus general population) criterion could eventually be studied by comparing these families with SAD with families without SAD. The last (state-independence) criterion indicates that persons with the disorder should display the endophenotype whether or not the illness is active (Gottesman & Gould, 2003). This could be studied by measuring the endophenotype at different time points within the same individuals.

Leiden Family Lab study

The goal of our Leiden Family Lab study was to delineate endophenotypes of SAD, by investigating the second (co-segregation) and third (heritability) criteria for endophenotypes. We included 'target participants' with SAD with their partner and children, as well as the siblings of these patients with their partner and children (Figure 1). At least one child of the target participants should have heightened symptoms of SAD. SAD was diagnosed by a psychiatrist or trained clinician based on a clinical interview and the Mini-Plus structured interview (Bauhuis, Jonker, Verdellen, Reynders, & Verbraak, 2013; Sheehan et al., 1998; Sheehan et al., 2010; Van Vliet & De Beurs, 2007). The target participant should be between 25 and 55 years of age, and his/her child with heightened symptoms of SAD should be living at home. Target participants with comorbid disorders other than anxiety or depression were excluded. Inclusion criteria for all family members were good comprehension of Dutch language, and age above 8 years.

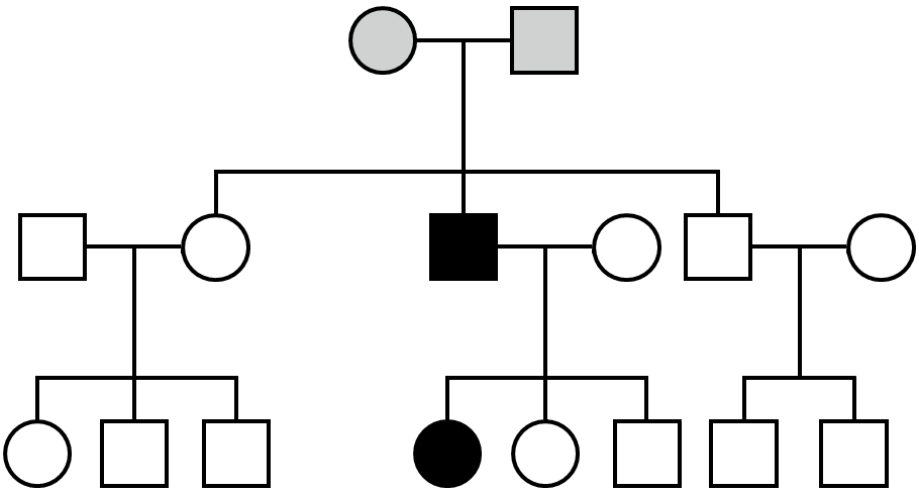

Figure 1. Example of a fictitious family in the Leiden Family Lab study on SAD. Families were selected based on two persons: an adult patient with SAD and his/her child with heightened symptoms of SAD. Grandparents (in grey) were not included.

Family members were asked to participate on one or two testing days in all parts of the Leiden Family Lab study: a clinical interview, an EEG session, an MRI session, questionnaires, and IQ measures (Figure 2). 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. Eventually, not all family members participated in all parts: some only filled out questionnaires at home, and some were not eligible to take part in the EEG or MRI sessions due to physical constraints, or epilepsy. This dissertation focuses on the EEG session. The MRI data is part of the dissertation of J.M. Bas-Hoogendam.

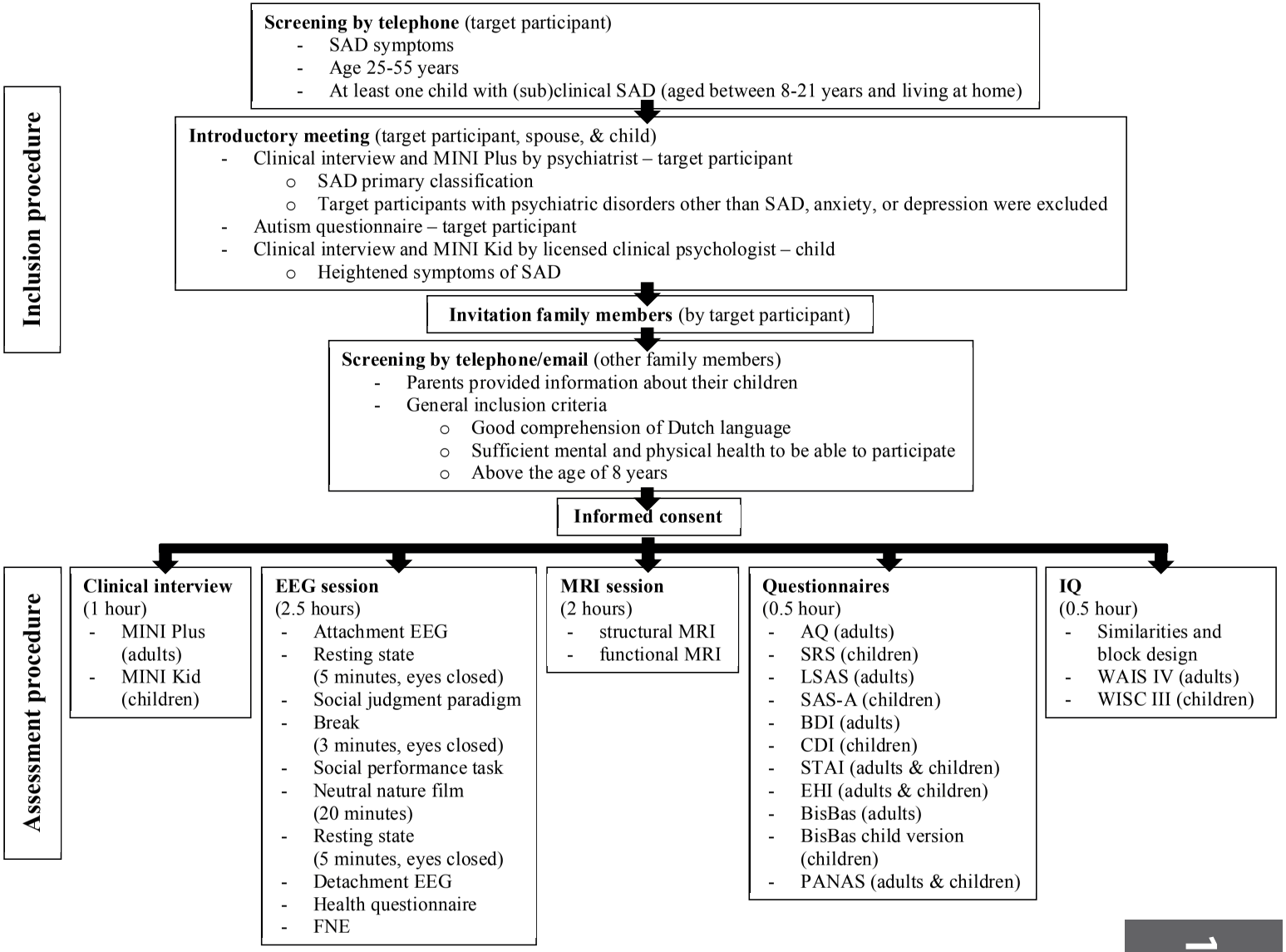
Inclusion procedure
Screening by telephone (target participant)
- SAD symptoms
- Age 25-55 years
- At least one child with (sub)clinical SAD (aged between 8-21 years and living at home)
Introductory meeting (target participant, spouse, & child)
- Clinical interview and MINI Plus by psychiatrist – target 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 Heightened symptoms of 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
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
- Break (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 2. Flow-chart of the inclusion and assessment procedures of the Leiden Family Lab study on SAD.

Note: 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, McCreary, Norton, & Asmundson, 2006); AQ = Autism-Spectrum Quotient Questionnaire (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 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, Steer, Ball, & Ranieri, 1996); CDI = Child Depression Inventory (Kovacs, 1992); STAI = State-Trait Anxiety Inventory (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 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, Meesters, De Kanter, & Timmerman, 2005); PANAS = Positive and Negative Affect Scale (Watson, Clark, & Tellegen, 1988); WAIS IV = Wechsler Adult Intelligence Scale IV (Wechsler, Coalson, & Raiford, 2008); WISC III = Wechsler Intelligence Scale for Children III (Wechsler, 1991).

Figure 3 shows an overview of the EEG session of the Leiden Family Lab study. EEG and heart rate were measured during resting state and during two tasks: the social performance task (Harrewijn, Van der Molen, & Westenberg, 2016) and the social judgment paradigm (Van der Molen et al., 2017; Van der Molen et al., 2014). We have chosen these tasks because they focus on one of the core features of SAD: fear of negative evaluation (APA, 2013; Clark & Wells, 1995; Rapee & Heimberg, 1997). In these tasks, feelings of social anxiety are elicited because participants have to give a speech in front of a video camera (social performance task) and because participants receive social feedback (social judgment paradigm). We studied several psychophysiological measures as putative endophenotypes of SAD: frontal alpha asymmetry, delta-beta cross-frequency correlation and heart rate variability during the social performance task, and N1, feedback-related negativity, P3 and theta power during the social judgment paradigm.

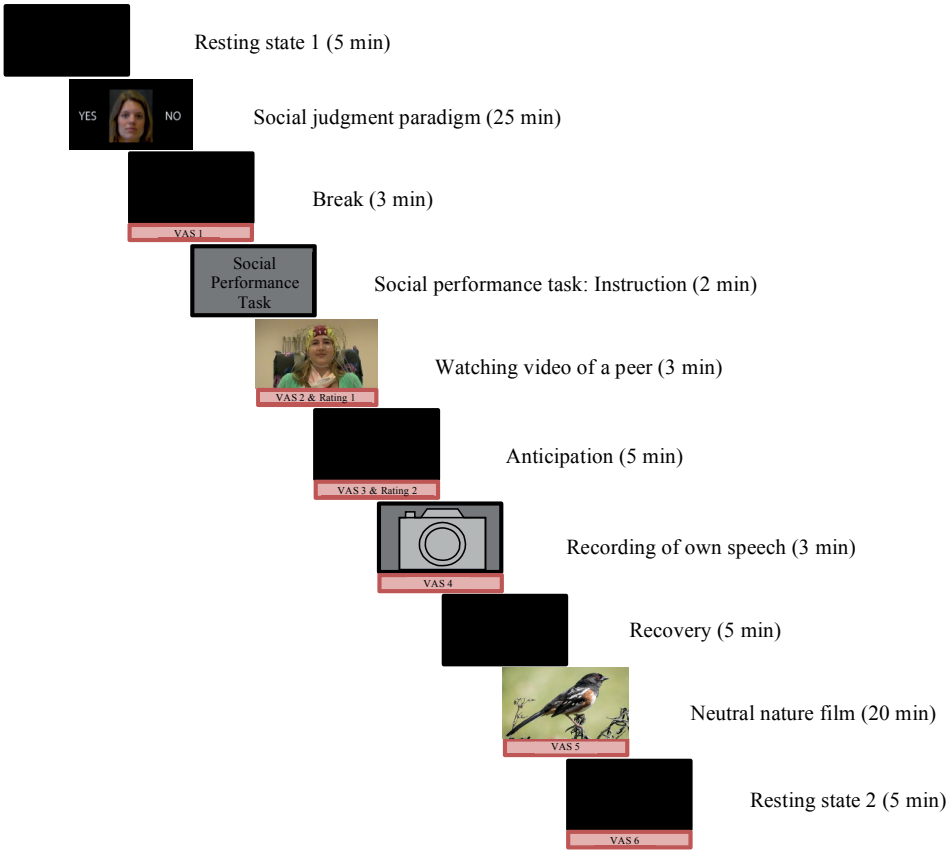

Figure 3. Overview of the EEG session. We asked participants to indicate their nervousness and avoidance at several time points throughout the EEG session on a visual analogue scale (VAS). Participants also evaluated the peer on the video (rating 1), and indicated how they expected to be evaluated (rating 2).

Outline of this dissertation

The goal of this dissertation was to delineate psychophysiological endophenotypes of SAD, to gain more insight in the mechanisms underlying the development and maintenance of SAD. The *second chapter* focuses on the first criterion (association) for endophenotypes by giving an overview of the most frequently studied EEG measures (both neural oscillatory power and event-related potentials) of information processing biases in SAD. The *third chapter* reports the validation of our newly developed social performance task in high and low socially anxious females. In this chapter we compare two commonly studied EEG measures in this task (frontal alpha asymmetry and delta-beta correlation). The other three chapters focus on

the second (co-segregation) and third (heritability) criteria for endophenotypes and describe the findings of our Leiden Family Lab study on SAD. In the *fourth chapter* we describe whether delta-beta correlation during the social performance task can be seen as a candidate endophenotype of SAD. The *fifth chapter* focuses on heart rate variability during resting state and the social performance task as a candidate endophenotype of SAD. The *sixth chapter* describes whether behavioral (i.e. expectations about social evaluation and corresponding reaction time) and EEG (i.e. N1, feedback-related negativity, P3, and theta power) measures in the social judgment paradigm can be seen as candidate endophenotypes. Finally, in the *seventh chapter* we discuss the results of this dissertation, and describe directions for future research and the clinical implications of these results.

Chapter 2

Electrocortical measures of information processing biases in social anxiety disorder: A review

This chapter is published as:

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Abstract

Social anxiety disorder (SAD) is characterized by information processing biases, however, their underlying neural mechanisms remain poorly understood. The goal of this review was to give a comprehensive overview of the most frequently studied EEG spectral and event-related potential (ERP) measures in social anxiety during rest, anticipation, stimulus processing, and recovery. A Web of Science search yielded 35 studies reporting on electrocortical measures in individuals with social anxiety or related constructs. Social anxiety was related to increased delta-beta cross-frequency correlation during anticipation and recovery, and information processing biases during early processing of faces (P1) and errors (error-related negativity). These electrocortical measures are discussed in relation to the persistent cycle of information processing biases maintaining SAD. Future research should further investigate the mechanisms of this persistent cycle and study the utility of electrocortical measures in early detection, prevention, treatment and endophenotype research.

Introduction

Social anxiety disorder (SAD) is a highly prevalent and debilitating disorder characterized by fear and avoidance of social or performance situations that might lead to scrutiny and/or negative evaluation by others (Rapee & Spence, 2004; Spence & Rapee, 2016). It is posited that social anxiety is expressed along a severity continuum (Rapee & Spence, 2004). That is, many people experience symptoms of social anxiety without meeting the clinical diagnostic criteria for SAD. When social anxiety symptoms hinder someone's daily-life functioning to such an extent that they avoid social situations, these people often meet the diagnostic criteria for SAD (APA, 2013). SAD is among the most prevalent psychiatric disorders, with a life-time prevalence ranging from 5.0% to 12.1% in the United States (Grant et al., 2005; Kessler et al., 2005). Patients with SAD have an increased risk for developing comorbid disorders, such as other anxiety disorders, depression, and substance abuse (Grant et al., 2005; Rapee & Spence, 2004; Spence & Rapee, 2016). Therefore, the identification of mechanisms underlying and maintaining SAD is of critical importance to improve (preventive) interventions for SAD.

Many cognitive-behavioral studies have demonstrated that information processing biases play an important role in the development and maintenance of SAD (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013; Wong & Rapee, 2016). Information processing biases might be displayed as biases in attention (e.g., hypervigilance, or self-focused attention) (Bögels & Mansell, 2004), interpretation (e.g., evaluating own behavior very critically, or interpreting social situations in a negative way), memory (e.g., selectively retrieving negative information), and imagery (e.g., experiencing images of oneself performing poorly in social situations) (Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013). Cognitive models posit that patients with SAD exhibit a persistent cycle of information processing biases, which perpetuate different stages of processing (i.e., automatic and controlled) and reinforce socially anxious behaviors over time. These information processing biases are triggered when the person is confronted with a socially stressful situation, repeated while in the situation, and carried forward in time when anticipating similar future events (Clark & McManus, 2002; Morrison & Heimberg, 2013). Electrocortical measures that are related to social anxiety could provide more insight in these information processing biases. So, to delineate electrocortical measures underlying the different stages of this persistent cycle of information processing biases, we reviewed EEG measures during rest, anticipation of, and

recovery from socially stressful situations, as well as event-related potential (ERP) measures during the processing of socially threatening stimuli.

We reviewed electrocortical measures of SAD, because EEG/ERP offers an online, objective and direct measure of brain activity. Of note, the future utility of potential electrocortical measures is highlighted by the relative ease of application and cost-effectiveness (Amodio et al., 2014; Luck, 2005). Most importantly, the high temporal precision of ERPs is very useful for capturing the precise timing of information processing biases during stimulus processing (Amodio et al., 2014; M. X. Cohen, 2011; Ibanez et al., 2012; Luck, 2005). The goal of this review was to provide a comprehensive overview of the most frequently studied EEG and ERP measures during rest, anticipation, stimulus processing, and recovery. These electrocortical measures may give insight into the mechanisms underlying and maintaining the persistent cycle of information processing biases in SAD, and might eventually be used in early detection, prevention, treatment and endophenotype research.

Focus

To delineate electrocortical measures related to the information processing biases in SAD, we reviewed studies that have reported on EEG spectral characteristics during rest, anticipation and recovery from a socially stressful situation, as well as ERPs during stimulus processing. Given that the social anxiety literature on EEG spectral characteristics has largely focused on power of the alpha frequency band and the correlation between the power of delta and beta frequency bands, these two EEG metrics were included in our review (Table 1). These EEG metrics were studied during resting state, in which participants sat still for a certain period of time, or during impromptu speech preparation tasks.

With respect to ERPs, studies on social anxiety have primarily investigated stimulus processing in face processing and in cognitive conflict paradigms. ERPs give precise insight in the timing of biases in processing of faces and errors/feedback. To put the ERPs into context and to show that differences in ERPs are not caused by differences in behavior, we also reported on behavioral findings in the tasks. Studies using face-processing paradigms typically include negative emotional faces as socially threatening stimuli because they communicate social dominance (Öhman, 1986) or disapproval for violated social rules or expectations (Averill, 1982, as discussed in Kolassa and Miltner, 2006). In this review, we further distinguished between explicit and implicit face processing paradigms (Table 2) to examine the effects of task-relevant (explicit) versus task-irrelevant (implicit) faces on the

modulation of early and late ERP components (Schulz et al., 2013). In explicit paradigms, participants are required to direct their attention to the emotional valence of stimuli. In implicit paradigms, participants are presented with emotional faces, but are required to direct their attention to different aspects of stimuli (e.g., indicating the gender of stimuli, or responding to a target replacing the faces). Our review focused on the early P1, N170, and P2 components, and the late P3 and late positive potential (LPP) components, since studies on social anxiety have examined these ERP components[1].

A recent and very relevant line of ERP research in social anxiety has focused on ERP components of feedback processing and performance monitoring in cognitive conflict paradigms. We reviewed ERP studies that have focused on the N2, feedback-related negativity (FRN), error-related negativity (ERN), correct response negativity (CRN), and positive error (Pe) components in these cognitive conflict paradigms (Table 3)[2].

We included studies reporting on patients diagnosed with SAD, as well as high socially anxious individuals, because both are expressions of social anxiety at the more severe end of the continuum (Rapee & Spence, 2004). We also reviewed studies examining constructs related to SAD, such as fear of negative evaluation, social withdrawal, shyness, and behavioral inhibition, since these constructs share common symptoms of SAD (Stein, Ono, Tajima, & Muller, 2004). Fear of negative evaluation is considered as a hallmark cognitive feature of SAD, whereas social anxiety is a more complete measure encompassing behavioral and affective symptoms (Carleton et al., 2006). Social withdrawal is a behavioral style commonly observed in childhood that is characterized by a lack of engagement in social situations or solitary behavior, such as playing alone (Rubin & Burgess, 2001). Shyness is a personality dimension defined as self-preoccupation and inhibition in social situations (Cheek & Buss, 1981). Behavioral inhibition is a temperament observed in infancy as negative reactivity to novel social and nonsocial stimuli (Hirshfeld-Becker et al., 2008). While these constructs are different, they are related to each other and to a greater risk of developing SAD (Clauss & Blackford, 2012; Hirshfeld-Becker et al., 2008; Stein et al., 2004).

We focused our review on studies of adults, due to several factors that hinder a comprehensive comparison between adult and child studies. For instance, brain development

[1] For studies using face processing paradigms, we did not report on the C1, N1, P150, N250, FN400, correct-response negativity (CRN), vertex positive potential (VPP), early posterior negativity (EPN), contralateral delay activity (CDA), and stimulus-preceding negativity (SPN) components, because very few (only 1 to 3) studies have investigated these components in relation to social anxiety.

[2] For studies using cognitive conflict paradigms, we excluded results on the N1, P150, P2, P3, LPP, CDA, and SPN components, because very few (only 1 to 2 studies) have reported on these components in social anxiety.

should be taken into account when comparing spectral EEG measures and ERPs between adults and children. Brain development is associated with a decline in total EEG power, as well as a shift from dominant slow wave (theta) activity to the dominant alpha rhythm as seen in adults (Marcuse et al., 2008; Segalowitz, Santesso, & Jetha, 2010). Such age-related differences in spontaneous EEG activity question the similarity in the functional significance of electrocortical measures when compared between age groups. Also, different methodological approaches might be required in quantifying these spectral measures (e.g., spectral band-width of alpha power should be different between young children and adults), which does not happen often in the literature. With regard to the ERP technique, comparing data between child and adult samples might be complicated by other factors, such as information processing efficiency, strategies used to allocate attention, and even task instructions (Segalowitz et al., 2010). Therefore, we focused mainly on electrocortical studies in adults, but we included a paragraph on developmental studies at the end of the review (Table 4 and 5).

This review is organized as follows: First, we describe briefly the information processing biases in social anxiety as recognized in the cognitive-behavioral literature. These cognitive-behavioral findings (e.g., attention biases, hyperviliance/avoidance tendencies) can be used as an information processing framework (Clark & McManus, 2002) for interpreting the electrocortical measures of SAD. Second, we give an introduction to EEG spectral characteristics and then review studies on spectral EEG analyses at rest, during anticipation of and recovery from socially stressful situations. Third, we introduce the ERP method, and review studies that report on early and late ERP components in response to facial stimuli and ERP components in cognitive conflict paradigms as potential indices of information processing biases in social anxiety. Lastly, we conclude by relating our findings to the persistent cycle of information processing biases that maintains SAD, and discussing the utility of electrocortical measures of SAD. We also describe current methodological challenges in electrocortical studies, and developmental studies involving these EEG and ERP measures of SAD.

Search strategy

We searched Web of Science for electrocortical studies in socially anxious individuals, using the key terms *EEG or ERP or oscillation** and *social anxi* or social anxiety disorder or fear of negative evaluation or social withdrawal or shy* or behavioral inhibition*, combined with *resting state, anticipation, recovery, face, stimulus processing, emotion, error, or*

performance monitoring. We also searched the reference list of the articles for additional studies, and searched for other publications of the authors of the articles. The data search was conducted before February 16th, 2017. The inclusion criteria for studies were including participants older than 18 years, who displayed SAD, high social anxiety, fear of negative evaluation, social withdrawal, shyness, or behavioral inhibition (as determined by standardized, validated measures). We included all published papers that were written in English. The data search resulted in a total of 35 studies.

Information processing biases in social anxiety

Cognitive-behavioral studies have repeatedly shown that socially anxious individuals display information processing biases in attention, interpretation, memory, and imagery (for extensive reviews, see Bögels and Mansell, 2004; Clark and McManus, 2002; Heinrich and Hofmann, 2001; Hirsh and Clark, 2004). These information processing biases can occur before, during, and after social situations (Hirsch & Clark, 2004).

Prior to a social situation, socially anxious individuals may exhibit information processing biases because they anticipate that negative events might result from the social encounter (Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004). An example of a socially stressful situation is public speaking. Research has shown that feelings of anxiety can be evoked in anticipation of performing a public speech (Westenberg et al., 2009). This anticipatory anxiety enhances perceptual processing and directs attention to socially threatening stimuli such as emotional faces (Wieser, Pauli, Reicherts, & Muhlberger, 2010). During the anticipation of a socially stressful situation, socially anxious individuals display memory biases. For example, high socially anxious individuals selectively retrieved negative impressions about oneself, and patients with SAD selectively retrieved past social failures (Clark & McManus, 2002). Patients with SAD estimated the chance of negative social events higher than controls or patients with other anxiety disorders (Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004). Furthermore, patients with SAD estimated the consequences of negative social events and evaluation by others as more severe than controls or patients with other anxiety disorders (Hirsch & Clark, 2004).

Cognitive models posit that information processing biases during anticipation might steer attentional focus towards potentially threatening social cues (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013). This notion is in line with the hypervigilance-avoidance theory of

attentional function in anxiety disorders (Mogg et al., 1997). This theory states that socially anxious individuals process socially threatening stimuli in two stages: initial vigilance (i.e., allocating attention to threatening stimuli), followed by avoidance of these stimuli (after 500-1000 ms) (Bögels & Mansell, 2004; Mogg, Bradley, DeBono, & Painter, 1997).

These information processing biases impact the thoughts and beliefs in socially anxious individuals after such socially stressful situations, triggering post-event rumination. For example, shortly after a social situation, patients with SAD interpreted ambiguous social situations in a negative way, and mildly negative situations in a catastrophic way (Brozovich & Heimberg, 2008; Clark & McManus, 2002). Socially anxious individuals displayed a recall bias, they were more likely to remember past negative social situations (Brozovich & Heimberg, 2008; Clark & McManus, 2002). Further, socially anxious individuals displayed prolonged and more perseverative self-focused thoughts and negative interpretations of themselves after a socially stressful situation (Brozovich & Heimberg, 2008).

Although these information processing biases seem to be triggered by a socially stressful situation, there is also evidence suggesting that information processing biases occur spontaneously, and hence are not restricted to a specific social situation. However, because there is no overt behavioral response linked to spontaneous information processing biases, much of this research stems from studies of "intrinsic" measures of brain functioning during rest, which are thought to reflect a history of brain activation in goal-directed, purposeful processing states (Sylvester et al., 2012). Indeed, resting-state functional MRI (fMRI) studies have shown that social anxiety was related to an imbalance between the amygdala and prefrontal cortex, which is linked to emotion dysregulation (Miskovic & Schmidt, 2012). Moreover, some EEG studies have shown social anxiety is related to differential resting brain activity linked to negative emotion and withdrawal-related social behaviors (Miskovic, Moscovitch, et al., 2011; Schmidt, 1999).

Together, there is accumulating evidence from cognitive-behavioral studies suggesting that socially anxious individuals display information processing biases during various contexts. Although these studies have offered important insights into the characteristics of information processing biases, they were not able to delineate the exact nature and time-course of these biases. This is mainly due to constraints of subjective dependent variables (e.g., self-report data), as well as a limitation in isolating specific processes (e.g., stimulus detection, categorization, response selection). Electrocortical studies provide a direct and objective index of information processing with high temporal resolution (Amodio et al., 2014; M. X. Cohen, 2011; Kotchoubey, 2006; Luck, 2005), and could yield a richer understanding

of how social anxiety is maintained. Such results could provide valuable insight in unraveling disorder-specific biological measures that in turn could facilitate early diagnosis and (preventive) intervention.

Spectral EEG measures related to information processing biases in social anxiety

The degree of synchronous firing of pyramidal neurons measured at the scalp with EEG is reflected in neuronal oscillations of different frequencies (Knyazev, 2007; Von Stein & Sarnthein, 2000). The range of frequencies in the human EEG that are typically examined in electrocortical studies include the delta (1 to 3 Hz), theta (4 to 8 Hz), alpha (8 to 13 Hz), beta (13 to 30 Hz), and gamma (30 to 100 Hz) bands. Rhythmic changes in the strength of oscillatory activity in a certain frequency band can be induced by various mental operations, and is reflective of different brain functions (Knyazev, 2007). In addition, the cross-talk between low and high EEG frequency bands – represented by indices of amplitude-amplitude or phase-amplitude coupling – have been suggested to reflect the functional communication between distant brain regions (Bastiaansen, Mazaheri, & Jensen, 2012; Schutter & Knyazev, 2012). In the social anxiety literature, researchers have mainly focused on alpha power, and the correlation between delta and beta power. Thus, our review is limited to these spectral EEG measures (Table 1).

Frontal alpha asymmetry

An influential theory on hemispheric asymmetry and emotion suggests that individual differences in positive and negative affect can be quantified in terms of asymmetry patterns in frontal alpha power (Davidson, 1992, 1998). More specifically, relatively greater left frontal cortical activity is related to approach behavior, whereas relatively greater right frontal cortical activity is related to withdraw behavior (Davidson, 1992, 1998). However, it should be noted that there is no simple correspondence between positive/negative affect and approach/avoidance behavior. For example, anger is a negative emotion related to approach behavior and was also related greater left frontal cortical activity (Harmon-Jones & Allen, 1998; Harmon-Jones, Gable, & Peterson, 2010). Frontal alpha asymmetry is typically measured by subtracting log-transformed left lateralized frontal alpha power from log-transformed right lateralized frontal alpha power (Allen, Coan, & Nazarian, 2004). Since alpha power is inversely related to cortical activity, positive alpha asymmetry scores reflect

relatively greater left frontal cortical activity (i.e., decreased left frontal alpha power), and negative alpha asymmetry scores reflect relatively greater right frontal cortical activity (i.e., decreased right frontal alpha power) (Allen et al., 2004). Frontal alpha asymmetry has been examined in relation to the behavioral approach and avoidance systems (Carver and White, 1994). Some studies have shown that right frontal alpha asymmetry is related to behavioral inhibition (Coan & Allen, 2004), whereas other studies have shown that this relation is more complex and not related to behavioral inhibition alone (Coan & Allen, 2003).

Frontal alpha asymmetry in social anxiety

Rest. Frontal alpha asymmetry has often been studied during resting state EEG measurements (or baseline), in which participants are asked to sit still during a certain period of time, with their eyes open or closed. The literature on frontal alpha asymmetry during resting state in social anxiety appears to be mixed. For example, patients with SAD showed increased left frontal activity after cognitive-behavioral therapy (Moscovitch et al., 2011). However, this study did not include a control group nor a treatment control condition, so it cannot be concluded that SAD patients showed increased right frontal activity compared to controls before treatment. Frontal alpha asymmetry during resting state has also been investigated in relation to constructs related to social anxiety, such as shyness in nonclinical samples. For example, greater right frontal activity has been observed in adults scoring high on shyness versus those scoring low on shyness (Schmidt, 1999). In contrast, other studies have found no difference in resting frontal alpha asymmetry between patients with SAD and controls (Davidson, Marshall, Tomarken, & Henriques, 2000), between high and low socially anxious individuals (Beaton et al., 2008; Harrewijn et al., 2016), and between high and low socially withdrawn individuals (Cole, Zapp, Nelson, & Perez-Edgar, 2012).

Anticipation. Cognitive models have highlighted the importance of information processing biases when socially anxious individuals anticipate exposure to feared social situations. Patients with SAD typically anticipate a more negative outcome in social situations and have more negative expectations about their own performance in social situations. Patients with SAD fear behaving in an inappropriate way, because it might result in negative evaluation by others (Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004).

Typically, anticipatory anxiety in SAD is examined via impromptu speech preparation tasks, in which participants are asked to prepare a speech on a general topic or on personal

characteristics. An example of a social performance task is presented in Figure 1. Some studies have shown that frontal alpha asymmetry is related to social anxiety during anticipation in such socially stressful situations (Cole et al., 2012; Davidson et al., 2000). For example, Davidson et al. (2000) examined frontal alpha asymmetry in patients with SAD while they were anticipating to perform a speech about an unknown topic and while preparing this speech when they were informed about the topic. Patients with SAD showed increased right anterior temporal activity during anticipation and planning compared to resting state (Davidson et al., 2000). Likewise, high socially withdrawn individuals showed increased right frontal activity during anticipation of performing their own speech, when they watched a video of a confederate talking in an anxious way, but not when the confederate talked in a non-anxious way (Cole et al., 2012). Other studies have found no effect of social anxiety between high versus low socially anxious individuals during anticipation of a speech (Beaton et al., 2008; Harrewijn et al., 2016), or between high versus low shy individuals during anticipation of a social interaction (Schmidt & Fox, 1994). Although Beaton et al. (2008) did not find a difference between high and low socially anxious individuals, shyness was related to increased right frontal activity in their sample, but only after controlling for depression.

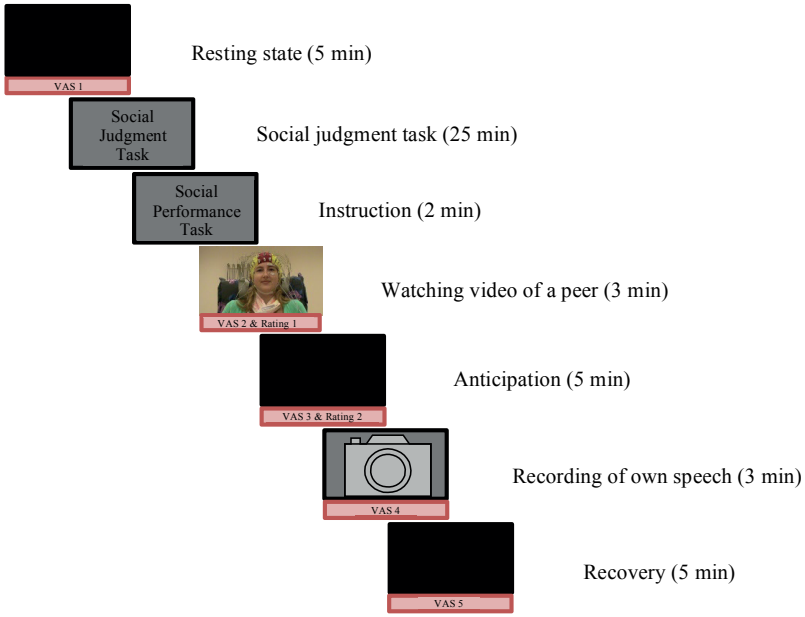

Figure 1. Example of a social performance task. This task includes a recovery phase after giving the speech, which is a novel compared to usual designs that measure only resting state and anticipation.

The mixed findings among these studies can be explained in several ways. First, the effect of social anxiety might only be measurable at extreme levels of social anxiety. That is, the effect was significant for patients with SAD (Davidson et al., 2000), who presumably experience more social anxiety, than high socially anxious individuals. However, the sample size in the study of Davidson et al. (2000) was rather small (14 patients with SAD), and thus these results need to be interpreted with caution. Furthermore, Cole et al. (2012) only found increased right frontal activity in high socially withdrawn individuals in the anxious condition. Tasks without such an anxiety-inducing condition might not elicit an increase in frontal alpha asymmetry, such as in Harrewijn et al. (2016). Second, the effect of social anxiety might only be measurable if the control group shows no anxiety during the task. For example, control participants in the study of Davidson et al. (2000) showed no increase in subjective anxiety during anticipation, whereas low socially anxious participants in the study of Harrewijn et al. (2016) showed an increase in subjective anxiety. An increase in subjective

anxiety in control participants might render the inability to detect significant group differences in frontal alpha asymmetry. Third, Davidson et al. (2000) focused on the difference between anticipation and resting state, whereas most studies only focused on anticipation (Beaton et al., 2008; Cole et al., 2012; Harrewijn et al., 2016; Schmidt & Fox, 1994). However, no effect of social anxiety was found when analyzing the difference between anticipation and resting state data in the Harrewijn et al. (2016) study. Fourth, the effect of social anxiety on frontal alpha asymmetry during anticipation might also be related to differences in the duration of the anticipation period. Studies that did not find frontal alpha asymmetry effects (Harrewijn et al., 2016; Schmidt & Fox, 1994) used relatively longer anticipation periods (i.e., 5-6 minutes) compared to studies that used shorter anticipation periods (Beaton et al., 2008; Cole et al., 2012). Particularly, Davidson et al. (2000) used an anticipation period of 3 minutes and a planning condition of 2 minutes that presented new information (topic of the speech), which might have increased participants' anxiety again during this phase. Overall, null effects in studies that have employed longer anticipation periods might be due to a habituation effect. That is, if the anticipation period is longer, participants' anxiety might habituate and less right frontal activity is shown towards the end. Possible habituation effects should be examined in future studies by comparing frontal alpha asymmetry of various time-bins during the anticipation period.

Recovery. Recovery from a socially stressful situation, such as performing a speech, might induce increased post-event processing in socially anxious individuals. According to various cognitive-behavioral studies (Brozovich & Heimberg, 2008; Clark & McManus, 2002), post-event processing in social anxiety is characterized by rumination and perseverative thinking (e.g., negative beliefs about past performance during a social situation). This enhanced retrieval of negative memories and a focus on negative assumptions are believed to maintain social anxiety symptoms (Brozovich & Heimberg, 2008). Potentially, post-event processing during recovery stages of a social performance task might be tracked by frontal alpha asymmetry. Only two studies have measured frontal alpha asymmetry during recovery from giving a speech. These studies failed to detect differences in frontal alpha asymmetry between patients with SAD and controls (Davidson et al., 2000) and between high and low socially anxious individuals (Harrewijn et al., 2016). Although the apparent scarcity of studies should be taken into account, these studies suggest that post-event processing in social anxiety is not reflected in patterns of frontal alpha asymmetry.

Delta-beta cross-frequency correlation

Another EEG metric that has been of interest in examining information processing biases in social anxiety during resting state, anticipation and recovery, is the cross-frequency correlation between the power (i.e., amplitude) of delta and beta oscillations, hereafter referred to as delta-beta correlation. Although different metrics of cross-frequency coupling exist, such as phase-phase or phase-amplitude coupling (M. X. Cohen, 2014), our focus is on the amplitude-amplitude coupling between the delta and beta frequency bands since this is the only metric that has been used in the social anxiety literature. We reviewed studies that have employed a similar experimental design as reviewed for the frontal alpha asymmetry studies (e.g., comparing resting state, as well as activity during anticipation of and recovery from a socially stressful situation).

Neural oscillations in the delta frequency range (1 to 3 Hz) are slow-wave oscillations that are hypothesized to stem from subcortical regions, whereas neural oscillations in the beta range (13 to 30 Hz) are fast-wave oscillations that are hypothesized to stem from cortical regions (Miskovic, Moscovitch, et al., 2011; Putman, Arias-Garcia, Pantazi, & Van Schie, 2012; Schutter & Knyazev, 2012; Schutter, Leitner, Kenemans, & Van Honk, 2006; Schutter & Van Honk, 2005; Velikova et al., 2010). It is posited that the cross-frequency correlation between slow- and fast-wave oscillations acts as an electrophysiological signature of the crosstalk between cortical and subcortical brain regions (Schutter & Knyazev, 2012). This is endorsed by a source localization analysis revealing that delta-beta correlation is associated with activity in the orbitofrontal and anterior cingulate cortex (Knyazev, 2011). Several studies have shown that positive delta-beta correlation is increased in anxious states, and interpreted this as increased communication between cortical and subcortical brain regions (Schutter & Knyazev, 2012). Delta-beta correlation was increased in anxiogenic situations in individuals scoring both high and low on general anxiety (Knyazev, Schutter, & Van Honk, 2006). Another study showed that participants with the largest increase in positive delta-beta correlation in an anxiogenic situation, also tended to have higher state anxiety scores (Knyazev, 2011). In contrast, Putman (2011) found no relation between delta-beta correlation and behavioral inhibition. So, some caution in interpreting delta-beta correlation is warranted, because there are some contradicting results, most research comes from one research group, the functional role of amplitude-amplitude coupling is unclear (Canolty & Knight, 2010), and it could be debated whether delta power solely reflects subcortical activity (Amzica & Steriade, 2000; Blaeser, Connors, & Nurmikko, 2017; Harmony, 2013).

Delta-beta cross-frequency correlation in social anxiety

Rest. The findings about delta-beta correlation at rest are mixed. Miskovic, Moscovitch, et al. (2011) showed that delta-beta correlation before cognitive-behavioral treatment was higher than after treatment in patients with SAD. However, when pretreatment delta-beta correlation of patients with SAD was post hoc compared with controls, there was no difference (Miskovic, Moscovitch, et al., 2011). Delta-beta correlation was increased in high compared to low behaviorally inhibited males (Van Peer, Roelofs, & Spinhoven, 2008). In contrast, two studies have reported no differences between high and low socially anxious individuals (Harrewijn et al., 2016; Miskovic et al., 2010). Overall, despite the small amount of studies, it seems that delta-beta correlation during resting state is not related to social anxiety.

Anticipation. As an electrocortical measure of social anxiety, delta-beta correlation seems more promising when socially anxious individuals are anticipating a socially stressful situation. That is, patients with SAD displayed increased positive delta-beta correlation during anticipation before treatment compared to low socially anxious individuals (post hoc comparison). This increased positive delta-beta correlation during anticipation in patients with SAD decreased after cognitive-behavioral treatment, and there was no difference between patients with SAD after treatment and low socially anxious individuals (Miskovic, Moscovitch, et al., 2011). High socially anxious individuals also displayed increased positive delta-beta correlation during anticipation compared to low socially anxious individuals (Miskovic et al., 2010). Another study has found increased negative delta-beta correlation in high compared to low socially anxious individuals (Harrewijn et al., 2016). The authors argue that negative delta-beta correlation could still be interpreted as increased crosstalk between cortical and subcortical regions, only in a different direction. Negative delta-beta correlation possibly reflects the known imbalance between subcortical and cortical brain regions in general anxiety (Bishop, 2007), and more specifically in SAD (Bruhl, Delsignore, Komossa, & Weidt, 2014; Cremers, Veer, Spinhoven, Rombouts, Yarkoni, et al., 2015; Miskovic & Schmidt, 2012). Together, these studies highlight the potential of delta-beta correlation as a sensitive electrocortical measure of SAD when individuals are anticipating a socially stressful situation.

Recovery. Despite the importance of post-event processing in social anxiety, only one study has examined delta-beta correlation during recovery from a socially stressful situation.

In this study, Harrewijn et al. (2016) examined delta-beta correlation during recovery from giving a presentation about their positive and negative qualities. Results showed that high socially anxious individuals showed increased negative delta-beta correlation compared to low socially anxious individuals (Harrewijn et al., 2016). This effect was interpreted as reflecting the imbalance between cortical and subcortical regions during recovery (Harrewijn et al., 2016). This is in line with findings from cognitive-behavioral studies suggesting that socially anxious individuals engage in post-event rumination after a socially stressful situation (Brozovich & Heimberg, 2008; Clark & McManus, 2002). Thus, the addition of a recovery phase in social performance paradigms seems valuable, and future studies should validate whether delta-beta correlation during recovery is a possible electrocortical measure of SAD.

Discussion of spectral EEG measures

The studies reviewed above provide insight in the potential of frontal alpha asymmetry and delta-beta correlation as electrocortical measures of SAD. Based on the available studies, it seems that delta-beta correlation is more strongly associated with SAD, relative to frontal alpha asymmetry.

Frontal alpha asymmetry during resting state and recovery was not related to social anxiety. However, frontal alpha asymmetry during anticipation appears to be a possible electrocortical measure of SAD, but only when the anxiety is extreme. This might suggest that frontal alpha asymmetry is not a trait-measure of SAD, but might be related to SAD in certain highly stressful states. Thibodeau, Jorgensen, and Kim (2006) have suggested that the mixed findings in alpha asymmetry literature could be related to comorbidity with depression. Unfortunately, only few studies in social anxiety have reported on depression as well. Two studies with participants with high levels of depression revealed an effect of social anxiety on frontal alpha asymmetry (Moscovitch et al., 2011; Schmidt et al., 2012). Beaton et al. (2008) found the relation between frontal alpha asymmetry and shyness when controlling for concurrent depression. In contrast, there was no effect of social anxiety in a sample with low levels of depression (Harrewijn et al., 2016).

Delta-beta correlation during anticipation and recovery appears to be more promising as a electrocortical measure of SAD. Functionally, delta-beta correlation is suggested to reflect the crosstalk between cortical and subcortical regions that is related to anxiety (Knyazev, 2011; Knyazev et al., 2006; Schutter & Knyazev, 2012). Indeed, source-localization analyses have shown that delta-beta correlation was associated with activity in the orbitofrontal and anterior cingulate cortex (Knyazev, 2011). Increased delta-beta correlation

in social anxiety converges with fMRI studies that have found an imbalance between cortical and subcortical regions in general anxiety (Bishop, 2007), but also more specific in SAD (Bruhl et al., 2014; Cremers, Veer, Spinhoven, Rombouts, Yarkoni, et al., 2015; Miskovic & Schmidt, 2012). This imbalance between cortical and subcortical regions also concurs with information processing biases that are found in cognitive-behavioral studies (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004). For example, increased anticipatory anxiety could be related to increased amygdala activation (Miskovic & Schmidt, 2012). However, some caution in this interpretation is warranted because the exact functional role of amplitude-amplitude correlation remains unclear (Canolty & Knight, 2010), it could be debated whether delta power solely stems from subcortical regions (Amzica & Steriade, 2000; Blaeser et al., 2017; Harmony, 2013), and most studies are performed by one research group. So, research on the exact meaning of delta-beta correlation, and independent replication of this effect is necessary. The effects were found in anticipation and recovery, which suggests that a certain level of stress-induction, or an anxious state, is necessary to find electrocortical measures of SAD.

ERPs related to information processing biases in social anxiety

To delineate electrocortical measures of SAD that are directly related to stimulus processing in face processing and cognitive conflict paradigms, we focused on ERP studies. ERPs are electrical potential changes in the brain that are time-locked to a certain stimulus and offer fine-grained information about the temporal dynamics of information processing (Koivisto & Revonsuo, 2010; Luck, 2005). ERPs provide objective insights into very early and late stages of stimulus processing (Luck, 2005). ERPs that are elicited as early as 100 ms after stimulus presentation are presumably modulated by physical characteristics of the stimulus rather than cognition (Herrmann & Knight, 2001; Luck, 2005). However, highly salient stimuli or changes in the order of stimulus presentation have been known to influence these early ERP components, reflecting stimulus-driven or bottom-up effects on attention (Knudsen, 2007; Luck, 2005). Early components that have been most frequently studied in social anxiety are the P1, N170 and P2.

In contrast, late ERP components are less influenced by variations in the physical characteristics of a stimulus, and reflect post-perceptual processing related to stimulus categorization, response selection/activation, and emotional reactivity evoked by stimuli (Eimer & Driver, 2001; Hajcak, MacNamara, & Olvet, 2010). These late ERP components

mostly reflect top-down effects on attention (Luck, 2005), a process through which neuronal sensitivity to specific task-relevant stimuli is increased (Knudsen, 2007). Late components that have been frequently studied in social anxiety are the P3 and late positive potential (LPP).

Due to its ability to distinguish between these early and late processing stages, ERPs offer objective measures to examine information processing biases in social anxiety. Here we focused on ERP components that are elicited by explicit or implicit face processing (Table 2) and cognitive conflict (Table 3) paradigms.

Early ERP components in face processing paradigms

P1. The P1 is an early positive ERP component that peaks 90-110 ms after stimulus onset. The P1 was previously seen as a stimulus-driven response that is not influenced by intentions, goals, and tasks (Eimer & Driver, 2001; Luck, 2005). However, more recent studies show that attention does influence the P1, as amplitude of the P1 increases to stimuli in an attended location compared to stimuli in an unattended location (Luck & Kappenman, 2013). The effect of attention of the P1 is maximal at the lateral occipital lobe and has been associated with activation in the lateral occipitotemporal cortex (Luck & Kappenman, 2013). Moreover, P1 amplitudes are enhanced in response to emotional faces compared to neutral faces in healthy adults. This suggests that enhanced attention is recruited in response to threat-related stimuli, and might be related to activity in the extrastriate visual cortex as seen in fMRI studies (Vuilleumier & Pourtois, 2007).

In explicit tasks, in which attention to emotion is required to complete the task, increased P1 amplitude in response to faces seems to be related to social anxiety (Figure 2). Patients with SAD showed increased P1 amplitude in response to schematic faces (i.e., line drawings of faces with different emotional expressions) in an emotion identification task and in a modified Stroop task (Kolassa et al., 2009; Kolassa, Kolassa, Musial, & Miltner, 2007). Increased P1 amplitude in response to pictures of faces was found in high versus low socially anxious participants in a modified Stroop task and in an emotional oddball paradigm (Peschard, Philippot, Joassin, & Rossignol, 2013; Rossignol, Campanella, et al., 2012). In the emotional oddball paradigm, P1 amplitude was increased in response to emotional faces versus neutral faces in high socially anxious individuals, whereas in low socially anxious individuals P1 amplitude was increased only in response to angry faces (Rossignol, Campanella, et al., 2012). This result indicates that high socially anxious individuals show a global hypervigilance towards emotional faces (Rossignol, Campanella, et al., 2012). This increased P1 amplitude was not related to any behavioral measures.

Also, increased P1 amplitudes may not be specifically linked to social anxiety, since patients with spider phobia also showed increased P1 amplitude when identifying faces (Kolassa et al., 2009). Furthermore, high socially anxious individuals showed increased P1 amplitude in response to colored rectangles in a modified Stroop task (Peschard et al., 2013), which suggests that increased P1 amplitudes reflect a more generic novelty response rather than early allocation of attention towards faces.

The effect of group (SAD, spider phobia, healthy controls) on P1 amplitude just failed to reach significance in one study (Kolassa & Miltner, 2006). That is, P1 amplitude did not differ between patients with SAD, patients with spider phobia, and healthy controls in a modified Stroop task. However, scores on the fear survey schedule were positively related to P1 amplitude only in patients with SAD (Kolassa & Miltner, 2006). This might be a power issue in this study, since only 19 patients with SAD were included. Most studies have shown that social anxiety is related to increased P1 amplitude in response to emotional faces in explicit tasks.

In implicit tasks, in which attention is directed to stimulus characteristics other than the emotional valence, increased P1 amplitude also seems to be related to social anxiety (Figure 2). Patients with SAD showed increased P1 amplitude in response to angry-neutral face pairs in a dot probe task, which was interpreted as an early hypervigilance to angry faces (Mueller et al., 2009). Patients with SAD showed an increased P1 amplitude in response to angry and neutral faces compared to happy faces in a face learning task, whereas controls did not show this effect of emotion (Hagemann, Straube, & Schulz, 2016). This might have been an novelty effect, the P1 effect was only present when the faces were shown for the first time, there was no effect of social anxiety on the P1 if the faces were shown for the second time in the test phase of this learning task (Hagemann et al., 2016). In the implicit condition of a modified Stroop task, patients with SAD showed increased P1 amplitude in response to all faces, compared to patients with spider phobia and healthy controls (Kolassa et al., 2007). High socially anxious individuals showed increased P1 amplitude in response to all faces in a dot probe task (Helfinstein, White, Bar-Haim, & Fox, 2008). P1 amplitude was also increased in the implicit condition of a modified Stroop task in high compared to low socially anxious individuals (Peschard et al., 2013), and in a spatial cueing task in individuals with high compared to low fear of negative evaluation (Peschard et al., 2013; Rossignol, Philippot, Bissot, Rigoulot, & Campanella, 2012).

In contrast to previous studies, Rossignol, Fisch, Maurage, Joassin, and Philippot (2013) showed that high socially anxious participants had decreased P1 amplitude in response

to faces in an attention-shifting paradigm. One reason for this contrasting finding might be that the stimuli are less threatening in this task, because they used faces and bodily postures of artificial humans. Artificial humans might not convey the same social evaluative threat as real humans. Another reason might be that participants can direct less attention to the face or bodily posture in the study of Rossignol et al. (2013), because the cue has no function in the rest of the task. In most other studies, the faces indicated the location of the target in some trials (Helfinstein et al., 2008; Mueller et al., 2009; Peschard et al., 2013; Rossignol, Philippot, et al., 2012). Also, this contradicting finding might be related to the overall slower response to targets in high socially anxious individuals in this task, since most other studies did not find behavioral differences between individuals with and without social anxiety (Hagemann et al., 2016; Kolassa et al., 2007; Mueller et al., 2009; Peschard et al., 2013; Rossignol, Philippot, et al., 2012). Furthermore, Kolassa and Miltner (2006) found no difference in P1 amplitude between patients with SAD, patients with spider phobia and healthy controls in the implicit condition of a modified Stroop task. However, as discussed above, this might be due to low power. Taken together, the majority of the reviewed studies provide evidence that social anxiety is related to increased P1 amplitude in implicit tasks.

The abovementioned studies all examined the P1 component in response to faces with a direct gaze. However, averted gazes might also elicit atypical electrocortical responses in socially anxious individuals due to their ambiguous nature (Schmitz, Scheel, Rigon, Gross, & Blechert, 2012). High socially anxious individuals showed increased P1 amplitude in response to viewing averted faces, although this finding did not reach statistical significance (Schmitz et al., 2012), possibly because the averted gazes were not threatening enough to elicit responses in high socially anxious individuals.

Two studies have focused on the P1 component in response to targets replacing the facial stimuli to measure whether the initial hypervigilance was maintained or followed by avoidance. On the one hand, in a dot-probe task, Mueller et al. (2009) showed *decreased* P1 amplitude in response to targets, interpreted as reduced processing of emotionally salient locations at later stages of stimulus processing. On the other hand, in a spatial cueing task, Peschard et al. (2013) showed *increased* P1 amplitude in response to targets, interpreted as maintained attention to the location of emotional cues. These contradicting findings could be linked to different processing stages as there were timing differences between the two tasks. In addition, the task of Mueller et al. (2009) might require more attention, because participants had to compare the target with the fixation cross, instead of just responding to the

target as in Peschard et al. (2013). Future research should clarify the information processing biases in later phases of dot-probe or spatial cueing tasks.

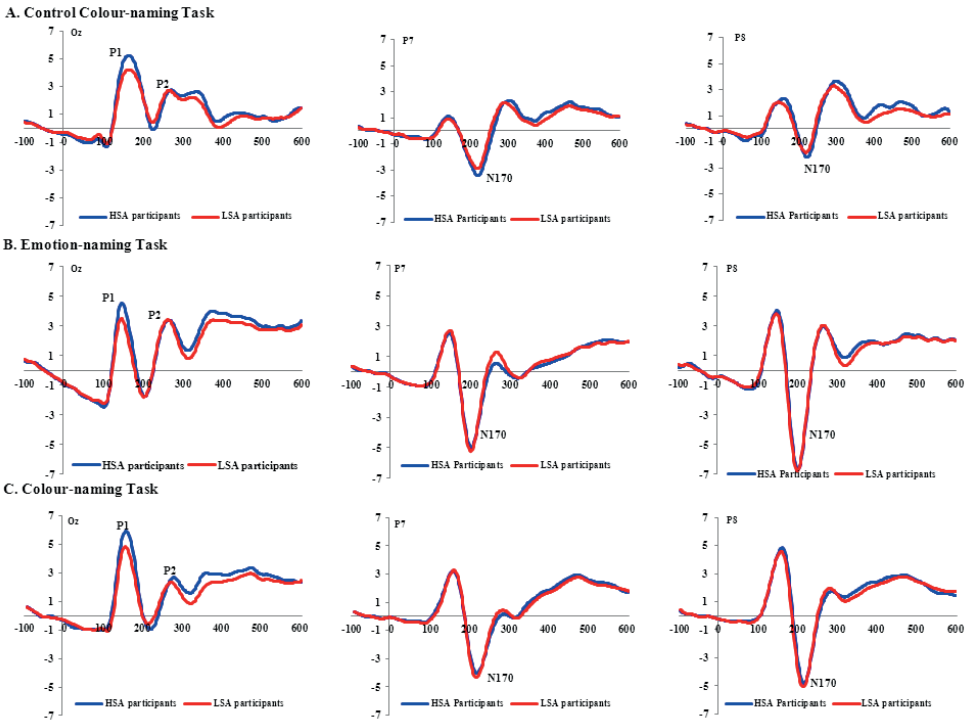

Figure 2. Social anxiety is related to increased P1 amplitude in response to explicit (emotion-naming task) and implicit tasks (color-naming task). High and low socially anxious individuals performed a modified Stroop task (3 conditions: color-naming of rectangles (A), emotion-naming of emotional faces (B), and color-naming of emotional faces (C)).

Reprinted from Biological Psychology, 93, Peschard, V., Philippot, P., Joassin, F, & Rossignol, M., The impact of the stimulus features and task instructions on facial processing in social anxiety: An ERP investigation, 88-96, Copyright (2013), with permission from Elsevier.

To conclude, most studies have shown that social anxiety is related to increased P1 amplitude. It should be noted that these studies have included relatively few participants (12 to 21 participants in the socially anxious groups), and the effect sizes are medium to high (η_p^2 ranging from 0.09 to 0.29). The relation between social anxiety and P1 amplitude is in line with the reviews of Staugaard (2010) and Schulz et al. (2013). The P1 is an early component that is mostly seen as a stimulus-driven or bottom-up response (Luck & Kappenman, 2013).

Increased P1 amplitude to emotional faces is suggested to reflect enhanced attention to threat-related stimuli (Vuilleumier & Pourtois, 2007). Given these functions of the P1, SAD might be related to information processing biases with underlying mechanisms linked to attention to threatening social stimuli in early phases of stimulus processing. Indeed, cognitive-behavioral studies have shown that SAD is related to hypervigilance to threatening stimuli (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013), and the P1 component might be the electrocortical measure of this early hypervigilance.

According to Jetha, Zheng, Schmidt, and Segalowitz (2012), the P1 component in response to emotional faces might be related to amygdala sensitivity to fear-related emotional faces. That is, the amygdala might have a causal role in fear processing as indexed by the P1 component (Rotshtein et al., 2010). The P1 component in response to fearful versus neutral faces was decreased in pre-operative patients with medial temporal lobe epilepsy, and patients with more severe amygdala damage showed lower P1 amplitudes (Rotshtein et al., 2010). In line with this hypothesis, fMRI studies in socially anxious individuals have shown increased amygdala activation in response to emotional faces (Miskovic & Schmidt, 2012; Schulz et al., 2013). So, this increased amygdala activation when viewing emotional faces, might be related to increased P1 amplitude. On the other hand, Mattavelli, Rosanova, Casali, Papagno, and Lauro (2016) showed that the medial prefrontal cortex influenced P1 amplitude during emotional face processing. They applied transcranial magnetic stimulation to the medial prefrontal cortex and found that P1-N1 amplitude in the right hemisphere decreased in response to happy and neutral faces (and not in fearful faces) during an explicit task. The authors suggested an early influence of top-down processing on face processing (Mattavelli et al., 2016). fMRI studies have also shown activation of the medial prefrontal cortex during face processing, albeit less substantial than amygdala activity (Miskovic & Schmidt, 2012; Schulz et al., 2013). Future research should clarify the influence of the amygdala and/or medial prefrontal cortex on P1 amplitude during face processing.

N170. The N170 is an early negative deflection in the ERP and is thought to measure early perceptual encoding and face categorization. The N170 peaks 130-200 ms after stimulus onset and is predominantly distributed at occipitotemporal electrodes (Luck, 2005; Pratt, 2013; Rossion & Jacques, 2013). Some studies have found that N170 amplitude is related to emotional expressions, whereas others have not found this sensitivity to emotion (for a review, see Vuilleumier & Pourtois, 2007). The functional role of the N170 in response to

faces is thought to underlie a full visual categorization, unlike the P1 that is thought to reflect rapid emotional processing based on crude visual cues (Vuilleumier & Pourtois, 2007).

In explicit tasks, the N170 does not seem to be modulated by social anxiety. Patients with SAD, patients with spider phobia and controls showed no differences in N170 amplitude in response to schematic faces in an emotion identification task and in a modified Stroop task (Kolassa et al., 2009; Kolassa et al., 2007). In response to pictures of emotional faces, N170 amplitude did not differ between high and low socially anxious participants in a modified Stroop task (Peschard et al., 2013) and in an emotional oddball paradigm (Rossignol, Campanella, et al., 2012). Only one study revealed increased N170 amplitude at right temporo-parietal electrodes when identifying angry faces in a modified Stroop task in patients with SAD compared to patients with spider phobia and healthy controls (Kolassa & Miltner, 2006). This contradicting finding could be caused by the use of more personal and ecologically valid stimuli in the study of Kolassa and Miltner (2006). They presented pictures of the entire face (Kolassa & Miltner, 2006), whereas other studies presented schematic (Kolassa et al., 2009; Kolassa et al., 2007) or trimmed faces without ears and hair (Peschard et al., 2013; Rossignol, Campanella, et al., 2012). However, most explicit tasks showed no influence of social anxiety on N170 amplitude.

N170 amplitude was also not modulated by social anxiety during tasks, in which participants' attention should be focused on stimulus characteristics other than emotion (implicit tasks). Patients with SAD showed no difference in N170 amplitude in the learning and test phases of a face learning task, compared to controls (Hagemann et al., 2016). Patients with SAD, patients with spider phobia and healthy controls also showed no difference in N170 amplitude in the implicit condition of a modified Stroop task with faces (Kolassa & Miltner, 2006), and with schematic faces (Kolassa et al., 2007). Studies reported no difference in N170 amplitude between high and low socially anxious individuals in an attention-shifting paradigm (Rossignol et al., 2013), in the implicit condition of a modified Stroop task (Peschard et al., 2013), and in a viewing task with direct and averted eye gazes (Schmitz et al., 2012), and between individuals with high and low fear of negative evaluation in a spatial cueing task (Peschard et al., 2013). Only one study contradicts this finding, by showing decreased N170 amplitude in patients with SAD in response to emotional faces in a dot-probe task (Mueller et al., 2009). However, they included only 12 patients with SAD, which might have been statistically underpowered (although the effect size was large, $\eta_p^2 = 0.20$). Furthermore, this dot-probe task was probably more difficult than the other dot-probe tasks, and therefore not comparable. That is, in Mueller et al. (2009), patients with SAD had to

compare the target with the fixation cross, instead of reporting on only one aspect of the target, such as the location, or direction (Peschard et al., 2013; Schmitz et al., 2012). Therefore, we conclude that social anxiety does not influence N170 amplitude in implicit tasks.

In sum, social anxiety is not related to N170 amplitude in both explicit and implicit face processing paradigms. Social anxiety also had no influence on behavioral performance in most of these studies. Only one study showed that high socially anxious individuals responded slower to the target than low socially anxious individuals in an attention-shifting paradigm (Rossignol et al., 2013). Patients with SAD and patients with spider phobia rated the angry schematic faces as more arousing, but they did not show differences in valence ratings, emotional classifications and reaction times (Kolassa et al., 2009). In his review, Staugaard (2010) concluded that differences between high socially anxious individuals and controls were mainly visible in the early P1 and N170 component. However, here we update this conclusion by showing that social anxiety is related to increased P1 amplitude, but not to changes in N170 amplitude, as most of the studies presented in the previous review of Staugaard (2010) were dated. Given that the N170 component in response to faces is not different between SAD and healthy controls, this implies that the N170 is not related to hypervigilance or threat detection strategies in socially anxious individuals.

P2. The P2 is a positive ERP component that peaks 150-250 ms after stimulus onset at anterior scalp sites (Luck, 2005). The P2 is an early electrocortical index of selective attention. That is, the P2 is increased in response to targets relative to non-targets or homogeneous stimuli. The P2 component is responsive to specific stimulus features, and is often increased in response to an infrequent target stimulus (Hajcak, Weinberg, MacNamara, & Foti, 2013; Luck, 2013). The P2 component is also associated with affective evaluation: P2 amplitude is typically increased in response to pleasant or unpleasant stimuli compared to neutral stimuli (Hajcak et al., 2013). Indeed, P2 amplitude was increased in response to emotional faces, which was interpreted as reflecting the rapid representation of emotional importance in prefrontal regions (Eimer & Holmes, 2007; Moser, Huppert, Duval, & Simons, 2008).

The P2 component seems to be unrelated to social anxiety when participants are asked to focus their attention on the emotional expression of a face. P2 amplitude did not differ between patients with SAD, patients with spider phobia and controls for happy, angry, and neutral faces in a modified Stroop task (Kolassa & Miltner, 2006), nor for schematic faces

that changed from neutral to gradually more angry, happy and sad faces in an emotion identification task (Kolassa et al., 2009). Furthermore, during a modified Stroop task, high socially anxious individuals did not differ in P2 amplitude from low socially anxious individuals (Peschard et al., 2013). Differences between high and low socially anxious individuals appeared only during a modified version of the Eriksen flanker task. Low socially anxious individuals displayed increased P2 amplitude in response to flankers consisting of happy or surprised compared to angry or disgusted faces, which was interpreted as a positive bias. High socially anxious individuals did not show this positive bias (Moser et al., 2008). However, it should be noted that this interaction was only significant at trend level (η_p^2 = 0.08), and was mainly driven by the effect in controls. In the other tasks, there was also no effect of emotion of the face in socially anxious individuals (Kolassa et al., 2009; Kolassa & Miltner, 2006; Peschard et al., 2013). The P2 results were unrelated to behavioral performance in these explicit tasks.

The results of implicit tasks on the relation between social anxiety and P2 amplitude are mixed. On one hand, in spatial cueing tasks, individuals with high fear of negative evaluation showed an increased P2 amplitude compared to individuals with low fear of negative evaluation in response to neutral, angry, disgusted, and happy faces (Rossignol, Philippot, et al., 2012), and in response to angry-neutral compared to fear-neutral face pairs (Peschard et al., 2013). Helfinstein et al. (2008) found a trend towards increased P2 amplitude in high compared to low socially anxious individuals in a dot-probe task. On the other hand, patients with SAD and controls showed no difference in P2 amplitude in the learning and testing phases of a face learning task (Hagemann et al., 2016). There was also no difference in P2 amplitude in the implicit condition of a modified Stroop task between patients with SAD, patients with spider phobia, and healthy controls (Kolassa & Miltner, 2006) and high and low socially anxious individuals (Peschard et al., 2013). In an attention-shifting paradigm with pictures of artificial humans (faces and bodily posture), Rossignol et al. (2013) found an overall decrease in P2 amplitude in high versus low socially anxious individuals. However, there was also no difference in P2 amplitude between high and low socially anxious individuals in a change detection task, though P2 amplitude was negatively correlated with task performance in self-focus trials in high socially anxious individuals (Judah, Grant, & Carlisle, 2016). Taken together, social anxiety was related to increased P2 amplitude in spatial cueing and dot-probe tasks (Helfinstein et al., 2008; Peschard et al., 2013; Rossignol, Philippot, et al., 2012), although these studies included only few participants (12-14 participants) in the socially anxious groups. Social anxiety was not related to increased P2

amplitude in attention-shifting, face learning, change detection and Stroop tasks (Hagemann et al., 2016; Judah, Grant, & Carlisle, 2016; Kolassa & Miltner, 2006; Peschard et al., 2013; Rossignol et al., 2013). Social anxiety is unrelated to task performance in most of these studies, with the exception that high socially anxious individuals respond slower to targets in the attention-shifting paradigm (Rossignol et al., 2013).

These findings suggest that the sensitivity of the P2 component as a measure of SAD seems to depend on explicit vs. implicit task instructions. During explicit tasks, there was no effect of social anxiety on P2 amplitude, suggesting that all participants mobilized their attentional resources to the same extent and showed the same level of emotional evaluation. However, in implicit spatial cueing and dot-probe tasks, individuals with social anxiety showed increased P2 amplitude, whereas individuals without social anxiety did not process the emotional faces when they were not required to. Functionally, the P2 component is an index of selective mobilization of attentional resources to certain stimuli (Hajcak et al., 2013; Luck, 2013). Thus, in specific implicit tasks, enhanced P2 amplitude might be related to an early emotional evaluation of affective stimuli. This coincides with information processing biases reported in cognitive-behavioral studies, which show that SAD is related to a focus on negative information (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004). Nevertheless, this effect should first be replicated in future studies with more participants.

Late ERP components in face processing paradigms

P3. The P3 is a positive deflection in the ERP typically observed 300-500 ms after stimulus onset and is distributed at frontocentral and centroparietal scalp sites (Hajcak et al., 2013; Polich, 2007). P3 amplitude is enhanced in response to infrequent targets in classic oddball paradigms, but is also sensitive to the amount of attention given to a stimulus (Luck & Kappenman, 2013; Polich, 2013). Polich (2007) proposed that the P3 comprises two subcomponents: the earlier component – P3a – has a frontocentral scalp topography, and is implicated in novelty detection (D. Friedman, Cycowicz, & Gaeta, 2001; Herrmann & Knight, 2001); the later component – the P3b – has a centroparietal scalp topography, and reflects the voluntary shift in attention towards target stimuli (Herrmann & Knight, 2001). According to Polich (2007), this 'family' of P3 components is thought to subserve a neural mechanism implicated in inhibiting extraneous brain activation to enhance the allocation of sufficient attentional resources during stimulus detection (P3a), and this process is guided by the contents of working memory specific to the task at hand (P3b). Emotional stimuli are also

known to modulate the P3 (Hajcak et al., 2013). In the social anxiety literature, the paradigms employed typically generated the P3b component (hereafter referred to as the P3), but when appropriate we distinguish between the P3a and P3b.

Most studies that have used explicit tasks to measure the P3 component have found no effect of social anxiety. For instance, there was no difference in P3 amplitude between patients with SAD, patients with spider phobia and controls in response to schematic faces in a modified Stroop task (Kolassa et al., 2007). There was also no difference in P3 amplitude between high and low socially anxious individuals in an emotional oddball task (Rossignol, Campanella, et al., 2012). These two studies showed no effect of social anxiety on behavioral performances. In addition, P3 amplitude did not differ between individuals with high and low fear of negative evaluation in an identification task (Rossignol, Anselme, Vermeulen, Philippot, & Campanella, 2007), and between high and low behaviorally inhibited males in an approach-avoidance task (Van Peer et al., 2007). However, social anxiety had an influence on behavior in these tasks. Individuals with high fear of negative evaluation detected disgusted faces before angry faces in all conditions, whereas individuals with low fear of negative evaluation did not show this differentiation (Rossignol et al., 2007). Individuals with high behavioral inhibition showed more state anxiety and tension during the task, but no differences in task performance (Van Peer et al., 2007). Only one study has found an effect of social anxiety on P3 amplitude in an emotional oddball task (Sewell, Palermo, Atkinson, & McArthur, 2008). That is, healthy participants were presented with happy, angry and neutral faces that were displayed in an upright and inverted position. Self-reported social anxiety was positively related to P3 amplitude in response to upright-presented, angry faces, suggesting an attentional bias towards processing threatening faces (Sewell et al., 2008). This contradicting finding might be related to task instructions to selectively focus on angry or happy faces, and analysis of only the unattended faces (Rossignol, Campanella, et al., 2012; Sewell et al., 2008). Taken together, it seems that social anxiety does not modulate the P3 component.

For implicit tasks, there seems to be no effect of social anxiety on P3 amplitude. P3 amplitude did not differ between patients with SAD and controls in the implicit condition of a modified Stroop task with schematic faces (Kolassa et al., 2007), nor between high and low socially anxious individuals in an attention-shifting paradigm (Rossignol et al., 2013), and individuals with high and low fear of negative evaluation in a spatial cueing task (Rossignol, Philippot, et al., 2012). Social anxiety affected task performance in the attention-shifting paradigm, showing that high socially anxious individuals responded overall slower to targets than low socially anxious individuals (Rossignol et al., 2013).

To conclude, there is no effect of social anxiety on the P3 component in explicit and implicit tasks, which corroborates prior discussion of the P3 in social anxiety (Staugaard, 2010). The P3 component is an index of the voluntary shift in attention towards target stimuli (Herrmann & Knight, 2001) and is also related to emotional content (Hajcak et al., 2013). The findings suggest that social anxiety is not related to an altered voluntary shift in attention, nor to aberrant processing of emotional content as indexed by the P3 component.

LPP. Studies that examined ERPs in response to the emotional content of stimuli have often found a positive deflection extending the traditional time-window of the P3. This component is coined the LPP, a sustained positive deflection that could last for seconds (Hajcak et al., 2013). The LPP is suggested to reflect the encoding and storage of intrinsically motivating stimuli, as it is larger after pleasant and unpleasant stimuli compared to neutral stimuli (Hajcak et al., 2010; Hajcak et al., 2013). Additionally, the LPP has been related to emotion regulation (Hajcak et al., 2010; Hajcak et al., 2013).

In explicit tasks, there are contradicting results regarding the LPP. For example, LPP amplitude was increased in angry or disgusted target faces in a modified version of the Erikson flanker task in high versus low socially anxious participants (Moser et al., 2008), whereas no difference in LPP amplitude was found in a modified Stroop task between patients with SAD, patients with spider phobia and controls in response to schematic faces (Kolassa et al., 2007). This difference might be related to arousal: Kolassa et al. (2007) used schematic stimuli that could be less arousing than real pictures, and Moser et al. (2008) showed 3 faces at the same time (a target face and two flanking faces) which could be more threatening for participants.

In an implicit face learning task, the LPP at a right central scalp site was increased in patients with SAD in response to learned versus novel faces task, but not in controls. However, this effect was the same for patients with SAD and controls in the left central or other parietal scalp sites (Hagemann et al., 2016). The LPP was also increased in response to faces with averted gaze compared to faces with direct gaze in high versus low socially anxious individuals (Schmitz et al., 2012). This result was interpreted to show the facilitated processing of negative stimuli during more detailed and sustained processing stages (Schmitz et al., 2012).

Most of these studies have found that social anxiety is related to an increased LPP, in absence of behavioral differences. This might suggest that social anxiety is related to increased processing of intrinsically motivating stimuli, and/or emotion regulation (Hajcak et

al., 2010; Hajcak et al., 2013). However, this suggestion should be confirmed in future studies since only few studies focused on the LPP in social anxiety and the effect sizes are medium (η_p^2 ranging from 0.07 to 0.13).

ERP components in cognitive conflict paradigms

A recent and very relevant line of ERP research in social anxiety has focused on ERP components that are related to feedback processing and conflict monitoring. In general, these studies assume that the socially anxious brain shows aberrant processing of cues that communicate performance errors or social rejection. Indeed, cognitive-behavioral studies revealed that socially anxious individuals are sensitive to signs that could convey social threat (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004). ERP components of interest are typically a class of medial-frontal negativities related to cognitive and attentional control, including the N2, FRN, ERN, and CRN, and the Pe (Gehring, Liu, Orr, & Carp, 2013; Van Noordt, Desjardins, & Segalowitz, 2015; Van Noordt & Segalowitz, 2012).

N2. The N2 is a negative component that peaks 200-350 m after stimulus presentation, and, depending on the task, has a frontocentral or centroparietal scalp distribution. It is proposed that the N2 component consists of at least three subcomponents: a frontocentral component associated with cognitive control, a frontocentral component associated with novelty or mismatch, and a posterior component associated with visual attention (Folstein & Van Petten, 2008).

First, the frontocentral N2 related to cognitive control did not differ between high and low socially anxious individuals in a modified version of the Eriksen flanker task (Moser et al., 2008), nor between individuals with high and low behavioral inhibition in a approach-avoidance task (Van Peer et al., 2007). The latter task showed increased state anxiety and tension in individuals with high behavioral inhibition, but no differences in task performance (Van Peer et al., 2007). Second, the frontocentral N2 related to novelty or mismatch was decreased in individuals with high fear of negative evaluation while detecting change in the intensity of anger during an emotional oddball task (Rossignol et al., 2007). Individuals with high fear of negative evaluation detected disgust before anger in all conditions, whereas individuals with low fear of negative evaluation did not show this pattern. However, it should be noted that only few individuals with high fear of negative evaluation (n = 10) participated (Rossignol et al., 2007). Third, the more posterior N2 component in response to the target

tone in a standard two-tone oddball paradigm was increased in patients with SAD compared to controls (Sachs et al., 2004). These few studies suggest that social anxiety is differentially related to various types of the N2 component, but this should be confirmed in future research.

FRN. The FRN is a frontocentral negative deflection peaking around 250-300 ms after a feedback stimulus (Gehring et al., 2013). The FRN component is increased when feedback is unexpected or reflects poor performance (Van Noordt & Segalowitz, 2012). However, recent studies showed that depending on the likelihood of an outcome, the FRN component might be sensitive to both negative and positive information (Ferdinand, Mecklinger, Kray, & Gehring, 2012; Oliveira, McDonald, & Goodman, 2007). Cao et al. (2015) found that patients with SAD displayed an increased FRN in response to acceptance feedback from peers. This was interpreted to reflect a violation of negative feedback expectancies, since socially anxious participants anticipated a larger proportion of negative peer feedback in this study (Cao et al., 2015). A difficulty with this interpretation is that expectancies were not recorded during the EEG experiment (on a trial-to-trial basis), but as an overall Likert-scale measure prior to the task to index general expectancies about the social evaluative outcome. Van der Molen et al. (2014) did measure participants' expectancy per trial during EEG recording, but did not find an association between the FRN and social anxiety. The FRN was only sensitive to feedback that violated participants' expectancies (Van der Molen et al., 2014). Further, the FRN did not differ in amplitude between high and low socially anxious individuals in trial-and-error learning task. There was only a marginal difference in FRN amplitude before learning between high and low socially anxious individuals when participants received false feedback about increased heart rate (to increase self-focus) (Judah, Grant, Frosio, et al., 2016). Taken together, studies have found mixed findings on the influence of social anxiety on the FRN component. A possible FRN effect might be related to the severity of symptoms, since the effect is significant in patients with SAD (Cao et al., 2015), marginally significant in high socially anxious individuals (Judah, Grant, Frosio, et al., 2016), and not significant in healthy participants (Van der Molen et al., 2014).

ERN. The ERN (or error negativity (Ne)) is a frontocentral negative deflection in the ERP that typically occurs about 50 ms after people make mistakes (Falkenstein, Hoormann, Christ, & Hohnsbein, 2000). Many studies have linked the ERN to activity in the anterior cingulate cortex (Holroyd & Coles, 2002; V. Van Veen & Carter, 2002; Yeung & Cohen,

2006), an important hub in the conflict monitoring network (Yeung & Cohen, 2006). Functionally, the ERN seems to reflect an error monitoring system, but it remains uncertain whether the ERN reflects a conscious or unconscious process of error detection (Wessel, 2012). It has been shown that the ERN is sensitive to motivational relevance of errors and individual differences in trait affect (M. J. Larson, Clayson, & Clawson, 2014). For example, ERN amplitudes are larger in individuals with perfectionistic or anxious tendencies, a finding that has been interpreted to reflect chronic conflict detection due to pathological worrying (Moser, Moran, & Jendrusina, 2012; Weinberg, Olvet, & Hajcak, 2010). In addition, the ERN is sensitive to social motivational factors, when performance is evaluated by others (Hajcak, Moser, Yeung, & Simons, 2005; Van Meel & Van Heijningen, 2010).

Patients with SAD showed an increased ERN compared to controls in a flanker task (see Figure 3) (Endrass, Riesel, Kathmann, & Buhlmann, 2014; Kujawa et al., 2016). An interesting finding was that the augmented ERN in SAD patients (children and adults) in the Kujawa et al. (2016) study persisted after SAD patients received treatment (i.e., cognitive-behavioral therapy or SSRI pharmacological treatment), suggesting these treatment options have little effect on desensitizing the error-detection mechanism in SAD. The ERN was also larger in high compared to low socially anxious individuals in a trial-and-error learning task, in which participants learned stimulus-response mappings (Judah, Grant, Frosio, et al., 2016). Sensitivity of the ERN to performance evaluation by a peer was recently shown in a study by Barker, Troller-Renfree, Pine, and Fox (2015). In this study, high and low socially anxious individuals performed a flanker task in two different conditions: alone or under peer observation. Results indicated that high socially anxious individuals showed larger ERN amplitudes when they were observed rather than when they were alone (Barker et al., 2015).

Several explanations have been offered for the increased ERN in SAD. For example, Kujawa et al. (2016) argued that patients with SAD monitor their own behavior more closely and are more sensitive to errors. This could be related to increased self-focused attention in social situations (Bögels & Mansell, 2004; Clark & McManus, 2002), but also to perfectionism as shown by the tendency to uphold high performance standards by patients with SAD (Clark & Wells, 1995). Alternatively, Moser, Moran, Schroder, Donnellan, and Yeung (2013) suggested that increased ERN amplitude in anxious apprehension might be related to processing inefficiency, caused by increased cognitive load, and increased compensatory mechanisms. Although this interpretation was not specific for SAD, it suggests that individuals with SAD are more distracted by their errors and need to use compensatory mechanisms. At the behavioral level, a candidate compensatory mechanism is post-error

slowing – a well-known increase in reaction time observed on the trial following an error (Danielmeier & Ullsperger, 2011; Gehring & Fencsik, 2001). Surprisingly, however, few studies have reported on post-error slowing in SAD, but the provisional evidence available suggests no significant differences in post-error slowing between SAD participants and controls (Endrass et al., 2014). Additionally, these reviewed ERN studies did not provide evidence of task performance differences (e.g., number of trials correct or % errors) between SAD and control participants, an observation that speaks to the notion that the augmented ERN in SAD might be reflecting a sensitive error-detection process, rather than an error compensation mechanism. However, examining behavioral measures such as post-error slowing in future ERN studies on SAD should validate this suggestion. Finally, it should be noted that only few studies have focused on the ERN component in relation to social anxiety, though the effect sizes are large for patients with SAD ($\eta_p^2 = 0.12$ in Kujawa et al. (2016) and $\eta_p^2 = 0.16$ in Endrass et al. (2014)) and medium for high socially anxious individuals ($\eta_p^2 = 0.08$ in Judah, Grant, Frosio, et al. (2016), and $\eta_p^2 = 0.11$ in Barker et al. (2015)). Thus, increased ERN amplitude appears to be a promising electrocortical measure of SAD.

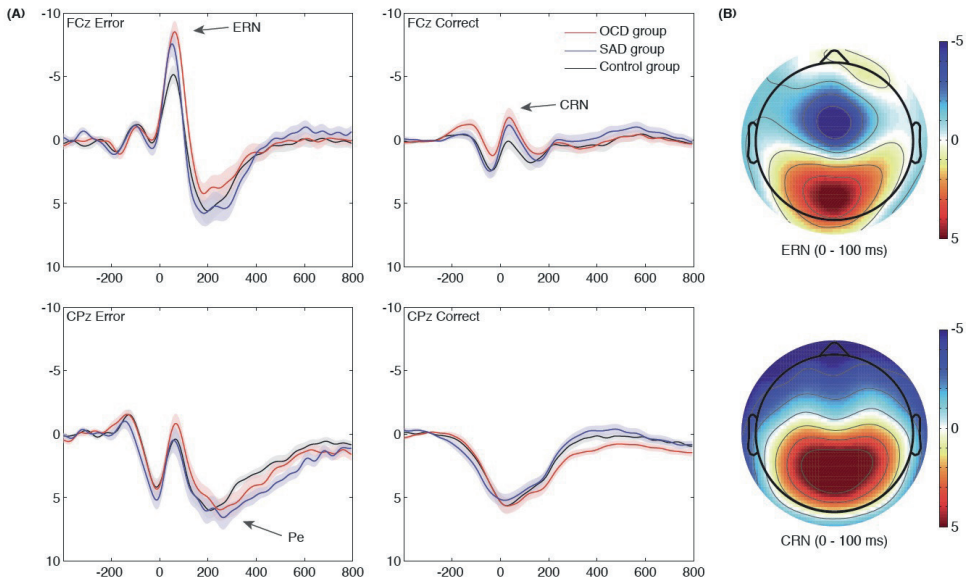

Figure 3. Social anxiety is related to increased ERN in patients with SAD and obsessive-compulsive disorder after errors in a flanker task.

Note: negative values are plotted upwards. Reprinted from Journal of Abnormal Psychology, 123, Endrass, T., Riesel, A., Kathman, N., & Buhlmann, U., Performance monitoring in obsessive-compulsive disorder and social anxiety disorder, 705-714, Copyright (2014), with permission from American Psychological Association.

CRN. The CRN is often studied concurrently with the ERN. The CRN resembles the ERN (negative deflection 50 ms after feedback), but is measured in response to correct rather than incorrect responses. The CRN component is usually smaller than the ERN component, but has a similar frontocentral scalp distribution (Gehring et al., 2013). Patients with SAD showed increased CRN amplitude in a flanker task (Endrass et al., 2014), and high socially anxious individuals showed increased CRN amplitude in a trial-and-error learning task (Judah, Grant, Frosio, et al., 2016). Moser et al. (2008) found no overall increased CRN amplitude in high socially anxious individuals. Nevertheless, high socially anxious individuals showed no difference in flanker interference effect in the CRN component between threatening and reassuring faces, whereas low socially individuals showed no flanker interference effect for threatening faces. This was interpreted as a positive bias that is lacking in high socially anxious individuals (Moser et al., 2008). In contrast, there was no difference in CRN amplitude between high and low socially anxious individuals in a flanker task performed alone nor when observed by a peer (Barker et al., 2015). Studies measuring both the ERN and CRN components have found that the effect of social anxiety on the ERN is larger than on the CRN (Barker et al., 2015; Endrass et al., 2014). Therefore, more studies are needed to draw conclusions about the possible influence of social anxiety on the CRN.

Pe. The Pe is also often studied in the same paradigms as the ERN and CRN. The Pe is a centroparietal, positive deflection 200-400 ms after an error, which might be related to an affective response, awareness, or adapting response strategies (Gehring et al., 2013). Most studies have shown no difference in Pe amplitude between patients with SAD and controls (Endrass et al., 2014) and between high and low socially anxious individuals (Barker et al., 2015) in flanker tasks. However, high socially anxious individuals showed marginally increased Pe amplitude compared to low socially individuals in a trial-and-error learning task. Furthermore, high socially anxious individuals showed a greater increase in Pe amplitude from trials before to after learning than low socially anxious individuals (Judah, Grant, Frosio, et al., 2016). The difference in these findings are probably related to the difference in tasks.

Discussion

The goal of this review was to give a comprehensive overview of the most frequently studied EEG spectral and ERP measures during rest, anticipation, stimulus processing, and recovery. Studies on EEG spectral characteristics have shown that delta-beta correlation during anticipation and recovery is a promising electrocortical measure, possibly reflecting the alleged imbalance between cortical and subcortical brain regions (Bishop, 2007; Bruhl et al., 2014; Cremers, Veer, Spinhoven, Rombouts, Yarkoni, et al., 2015; Miskovic & Schmidt, 2012). The ERP studies have shown information processing biases during early processing of faces and errors. Increased P1 amplitude in response to emotional faces is associated with social anxiety, reflecting hypervigilance to threatening stimuli (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013). Another electrocortical measure of SAD is increased ERN amplitude, possibly reflecting increased self-focused attention (Bögels & Mansell, 2004; Clark & McManus, 2002) or perfectionism (Clark & Wells, 1995). Finally, increased P2 amplitude was related to social anxiety, but only in implicit spatial cueing and dot-probe tasks. This might be related to a focus on negative evaluation as reported in cognitive-behavioral studies (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004). The reviewed studies did not provide evidence that frontal alpha asymmetry nor the N170, P3, LPP, N2, FRN, CRN and Pe components are electrocortical measures of SAD.

Cognitive-behavioral studies have proposed that SAD is maintained by a persistent cycle of information processing biases (Clark & McManus, 2002; Morrison & Heimberg, 2013). That is, attention biases are elicited by socially threatening stimuli, repeated while in the social situation, and carried forward over time by anticipation (Morrison & Heimberg, 2013). Indeed, we have shown that social anxiety is related to hypervigilance to threatening stimuli, such as faces and errors. Repetition within a social situation has not yet been studied, since ERPs are an average across multiple trials. The next step of the persistent cycle of information processing biases – carried forward over time by anticipation – has only partly been studied. We have found that social anxiety is related to increased delta-beta correlation during anticipation and recovery, but it is unknown whether this carries the attention biases forward over time and thus plays a role in the maintenance of SAD. Such a mechanism has been found in healthy participants, where anticipatory anxiety before giving a speech enhanced early ERP responses to angry faces (Wieser et al., 2010), but remains to be established SAD. Taken together, increased amplitudes of the P1 to faces and the ERN to

errors, and delta-beta correlation during anticipation and recovery might be possible electrocortical measures underlying the persistent cycle of information processing biases that maintains SAD. Future studies should investigate how hypervigilance is repeated within the situation, and whether is it carried forward over time during anticipation and recovery.

Another important avenue for future research is to investigate how these information processing biases are linked to behavior in patients with SAD. One important question is whether information processing biases during the early stages of stimulus processing (e.g. hyperviligance) trigger a cascade of biases during further processing stages. Most studies have focused on the ERPs individually, but it would also be important to know how the early biases influence later processing of stimuli. Another important question is how these information processing biases influence behavior. A promising field of research would be to examine whether ERN activity impacts subsequent decision-making (e.g., post-error slowing), which has only been scarcely studied in relation to SAD (Endrass et al., 2014). Future studies should continue this line of research in SAD, since such work would not only contribute to our understanding of information processing biases in SAD, but also to the psychological processes indexed by the ERN more generally. Another way of investigating the link with behavior is by using more ecologically valid paradigms, such as social performance tasks or social feedback tasks.

Electrocortical measures of SAD could be useful in research on early detection, prevention and treatment of SAD. Future studies should investigate whether amplitudes of the P1 and ERN, and delta-beta correlation can be used to identify persons at risk for developing SAD at a young age. Understanding the factors influencing the development of SAD in relation to functional brain development might be useful for developing preventive interventions. In addition, it would be valuable to know how such electrocortical measures could predict treatment response. For instance, it might be that persons who are sensitive to errors (those with an increased ERN component) need a different focus in treatment than persons who are displaying information processing biases during anticipation or recovery (those with increased delta-beta correlation). Recent studies with facial stimuli have shown that P1 amplitude might be a predictor of treatment outcome and N2 and LPP amplitudes might be predictors of treatment response in anxiety disorders (Bunford et al., 2017; Hum, Manassis, & Lewis, 2013). However, only a few electrocortical studies have focused on predicting treatment response in anxiety disorders (Lueken et al., 2016). Another interesting avenue for future research is to examine whether these electrocortical measures could help in unraveling the genetic basis of SAD. For example, these electrocortical measures can be

tested as possible endophenotypes of SAD (Glahn et al., 2007). This is a relatively new approach that has yielded promising results in depression and schizophrenia research (Bramon et al., 2005; Glahn et al., 2012; Glahn et al., 2007), and might be particularly fruitful in SAD research given the relatively high heritability (Isomura et al., 2015). Research on electrocortical measures of SAD should take the next step by validating these measures and studying how they could be used best to reduce social anxiety symptoms. In the following paragraphs, we discuss methodological and developmental considerations that should be addressed in future studies.

Methodological considerations

One issue that hampered delineating electrocortical measures of SAD is the diversity of experimental paradigms that have been used in the social anxiety literature. Furthermore, even when using similar paradigms, differences between ERP results can emerge due to the diversity in methodological strategies, such as ERP component scoring, filter and reference settings, the number of trials required to obtain the ERP of interest, and timing differences (J. Cohen & Polich, 1997; Hajcak et al., 2013). In addition, there are numerous inconsistencies in the names and definitions of electrocortical measures. For example, the often-used term 'cross-frequency coupling' could refer to different measures of electrocortical brain activity (Schutter & Knyazev, 2012). One of the challenges in cognitive electrophysiology is therefore to use unambiguous and consistent terminology (M. X. Cohen & Gulbinaite, 2014). It should also be noted that not all studies reported effect sizes, which makes it difficult to interpret and compare the effects of social anxiety across studies.

Future studies should also examine whether these electrocortical measures are specific to SAD. The studies reviewed above have focused mainly on participants with SAD or heightened symptoms of social anxiety. A few studies have already compared patients with SAD with patients with spider phobia as well as healthy controls (Kolassa et al., 2009; Kolassa et al., 2007; Kolassa & Miltner, 2006). However, specificity should also be studied by comparing patients with SAD and patients with other disorders that have a high comorbidity with SAD (such as generalized anxiety disorder or depression). Moreover, it should be investigated whether the electrocortical measures are specifically related to socially threatening stimuli (faces in most paradigms). Notably, high socially anxious individuals also displayed increased P1 amplitude in response to colored rectangles (Peschard et al., 2013), which questions the specificity of this electrocortical measure.

We have focused on constructs related to SAD, such as fear of negative evaluation, social withdrawal, shyness, and behavioral inhibition, because these constructs share common symptoms of SAD (Stein et al., 2004). However, some findings were only found in individuals characterized by these related constructs (e.g. the relation between shyness and right frontal cortical activity in Beaton et al. (2008)), which questions the generalizability of these findings to SAD. Given that not all shy and behaviorally inhibited individuals develop SAD (Spence & Rapee, 2016), future research should investigate which electrocortical measures are related to developing SAD. In addition, future research should also focus on the diagnostic utility of these electrocortical measures by investigating their specificity, sensitivity, and diagnostic value.

Developmental considerations

One of the objectives of examining electrocortical measures of SAD is to evaluate whether they can be used to detect individuals at risk for developing this debilitating disorder. Therefore, it is important to study these possible electrocortical measures in children. SAD has a relatively late onset and usually develops during early adolescence (Haller et al., 2014), and early detection of SAD in younger children typically involves the assessment of personality/temperamental constructs that have been interpreted as precursors of the disorder (e.g., behavioral inhibition and shyness). However, the key question is whether the EEG measures associated with behavioral inhibition or shyness are also related to SAD, since not all children with these related constructs eventually develop SAD (Spence & Rapee, 2016). In addition, the integration of findings from adult and child studies is complex due to age related differences in spontaneous EEG activity and the need for different methodological approaches. While being aware of these concerns, we here shortly describe electrocortical studies that have included children that might be at risk of developing SAD (Table 4 and 5).

With respect to frontal alpha asymmetry studies, the pattern of findings observed in children mimics the inconsistencies in the adult literature. For example, Fox et al. (2001) showed that children classified as behaviorally inhibited at 4 months exhibited increased right frontal activity at 9 and 14 months of age. In healthy children, increased right frontal activity was related to socially inhibited behavior (Henderson, Fox, & Rubin, 2001; Henderson, Marshall, Fox, & Rubin, 2004). In contrast, others did not find an association between frontal alpha asymmetry and SAD-related constructs, such as shyness (Schmidt et al., 1999; Theal-Honey and Schmidt, 2006) or social withdrawal (Fox et al., 1995; Hannesdottir et al., 2010).

Notably, in contrast to the adult studies reviewed earlier, there is no evidence of an early hypervigilance towards threatening stimuli or novelty in children (as indexed by early ERPs). For example, studies examining face processing in behaviorally inhibited children (Thai, Taber-Thomas, & Perez-Edgar, 2016), as well as novelty detection in an auditory oddball paradigm in behaviorally withdrawn children (Bar-Haim, Marshall, Fox, Schorr, & Gordon-Salant, 2003) did not find evidence of early hypervigilance as indexed by the early ERPs.

Developmental studies focusing on late ERPs revealed mixed results. Some studies found an enhanced LPP in children and adolescents with SAD to emotional faces (Kujawa, MacNamara, Fitzgerald, Monk, & Luan Phan, 2015), and a larger P3 to target and standard tones in shy children (Tang, Santesso, Segalowitz, & Schmidt, 2016). However, the novelty P3 was not associated with shyness (Tang et al., 2016), or behavioral inhibition in adolescence (Reeb-Sutherland et al., 2009). Although, a combination of high behavioral inhibition and high P3 amplitudes to novel sounds in adolescence, indicative of heightened attentional orienting, were more likely to have clinical anxiety diagnoses (Reeb-Sutherland et al., 2009).

Developmental studies of ERPs in cognitive conflict paradigms report mixed findings on the N2 component. Shyness did not affect N2 amplitude in a three-stimulus auditory oddball task (Tang et al., 2016), nor in a flanker task (Henderson, 2010). However, high behaviorally inhibited children showed increased N2 amplitude during a flanker task, and a combination of high behavioral inhibition and increased N2 amplitude predicted more withdrawal and less assertiveness in a social exclusion task (Lahat, Walker, et al., 2014). In addition, behavioral inhibition was related to social reticence at age 7 in children who showed increased N2 amplitude during a Go-NoGo task (Lamm et al., 2014). Shy children with increased N2 amplitudes reported higher levels of social anxiety (Henderson, 2010). In behaviorally inhibited children, N2 amplitude predicted a bias away from angry faces in a dot-probe task (Thai et al., 2016).

In terms of the FRN, mixed findings have been reported in developmental studies. For example, Lackner, Santesso, Dywan, Wade, and Segalowitz (2014) found that shyness was related to a decreased FRN to monetary feedback (no difference between wins or losses), whereas Kessel, Kujawa, Proudfit, and Klein (2015) reported an increased difference in FRN between wins and losses in social anxiety. Kujawa, Arfer, Klein, and Proudfit (2014) found that a greater difference in FRN between social acceptance and social rejection feedback was related to social anxiety.

ERN amplitude was the only electrocortical measure that was consistently found across adult and child studies. Behaviorally inhibited children (Lahat, Lamm, et al., 2014) and adolescents (McDermott et al., 2009) demonstrated a larger ERN in a flanker task, and increased ERN amplitude in behaviorally inhibited adolescents was related to a higher risk for anxiety disorders (McDermott et al., 2009). Furthermore, differences between ERN and correct-response negativity amplitude in 7-year-old children predicted SAD symptoms at age 9 (Lahat, Lamm, et al., 2014). It should be noted however that the ERN is not specific to SAD, but also found in other anxiety disorders in children (Wauthia & Rossignol, 2016). The CRN and Pe are each studied in only one developmental study and were not related to social anxiety (Lahat, Lamm, et al., 2014; McDermott et al., 2009).

Taken together, only the ERN component has been linked to social anxiety in both child and adult studies. This might suggest that the ERN could play a role in the early detection of SAD, although this should be confirmed in longitudinal studies. However, it should be noted that the studies in children and adults use different paradigms that render comparisons of the results and any long-term associations difficult. Accordingly, future studies should address the issues of measurement equivalence and adopt longitudinal designs to confirm the developmental associations. Nevertheless, these results speak to the importance of context to provide specificity in uncovering electrocortical measures of SAD. Contexts that involve social evaluation may be more salient for individuals who are socially anxious, particularly during adolescence – an important period for the development of SAD (Haller et al., 2014). Thus, brain functioning during social rejection or exclusion events in socially anxious individuals across development may provide more specific measures to understand the electrocortical mechanisms related to SAD.

Conclusion

In sum, social anxiety is related to delta-beta correlation during anticipation of and recovery from a socially stressful situation, increased P1 amplitude in response to processing emotional faces, and increased ERN amplitude after making errors. Together, these electrocortical measures might underlie the persistent cycle of information processing biases that maintains SAD. However, these electrocortical measures represent only a part of this persistent cycle, so future research should investigate repetition within the social situation and whether hypervigilance might be carried forward over time by information processing biases during anticipation and recovery. The influence of early ERPs on later ERPs and the link between

electrocortical measures and behavior should also be studied to gain more insight in the psycho(physio)logical mechanisms maintaining SAD. Given the abovementioned methodological and developmental concerns, we also call for studies that examine these electrocortical measures in larger samples using longitudinal designs. Such studies should validate these electrocortical measures and investigate whether these measures could (1) be identified at young age, (2) be used to prevent the development of SAD, (3) play a role in treatment of SAD (e.g. if they could predict treatment response), and (4) be seen as endophenotypes of SAD and thereby give insight in genetic mechanisms.

Table 1

Overview of studies about frontal alpha asymmetry and delta-beta correlation in social anxiety.

Author	**Participants**	**Sex ratio of target group (F:M)**	**Protocol**	**Behavioral results** (socially anxious relative to control)	**EEG Results** (socially anxious relative to control)
Frontal alpha asymmetry					
A. Resting state					
Moscovitch et al., 2011	Patients with SAD Pre and post CBT	11:12	Resting state	-	Increased left frontal activity after CBT (F3/4; $\eta_p^2 = 0.15$)
Schmidt, 1999	High-shy/high-social High-shy/low-social Low-shy/high-social Low-shy/low-social (extreme groups)	All 10:0	Resting state	-	Increased right frontal activity (F3/4)
B. Anticipation of and recovery from socially stressful situation					
Schmidt & Fox, 1994	High-shy/high-social High-shy/low-social Low-shy/high-social Low-shy/low-social (extreme groups)	All 10:0	Instruction Anticipation Social interaction	Low-social more socially anxious nonverbal behavior	No difference

Author	**Participants**	**Sex ratio of target group (F:M)**	**Protocol**	**Behavioral results** (socially anxious relative to control)	**EEG Results** (socially anxious relative to control)
C. Combined studies (both resting state and anticipation/recovery of/from socially stressful situation					
Davidson et al., 2000	Patients with SAD vs controls	14 patients with SAD (ratio unclear)	Resting state Instruction Anticipation Planning Speech Recovery	More anxiety in each condition, increase in anxiety during anticipation	RS: No difference ANT: Increase in right anterior temporal activity from resting state to anticipation and from resting state to planning (T3/4), same for lateral frontal activity (F7/8)* REC: No difference
Beaton et al., 2008	HSA vs LSA participants (extreme groups)	19:5	Resting state Instruction Anticipation Speech	-	RS: No difference ANT: No difference. After controlling for depression, only shyness was related to increased right frontal activity

Harrewijn et al., 2016	HSA vs LSA participants (extreme groups)	23:0	Resting state Social judgment task Instruction Watch video Anticipation Speech Recovery	More nervous at each time point (except baseline); more avoidance after video	RS: No difference (r = -0.12) ANT: No difference (r = -0.06) REC: No difference (r = -0.03)
Cole et al., 2012	High vs low socially withdrawn participants (median split)	12:9	Resting state Instruction Watch video Anticipation Speech	No influence on performance rating	RS: No difference ANT: After watching anxious video, increased right frontal activity during watching video and anticipation (F3/4; d = 0.81)
Delta-beta correlation					
A. Resting state					
Van Peer et al., 2008	High vs low behaviorally inhibited participants (extreme groups)	0:20	Resting state Cortisol vs placebo administration	-	Increased positive delta-beta correlation Delta-beta correlation increased after cortisol administration in both groups (Fz)

Author	**Participants**	**Sex ratio of target group (F:M)**	**Protocol**	**Behavioral results** (socially anxious relative to control)	**EEG Results** (socially anxious relative to control)
B. Combined studies (both resting state and anticipation/recovery of/from socially stressful situation					
Miskovic et al., 2011	Patients with SAD Pretreatment 1 Pretreatment 2 Midtreatment Posttreatment	12:13	Resting state Anticipation Speech	More anxiety during anticipation than resting state	RS: Decreased positive delta-beta correlation from pretreatment to midtreatment and from pretreatment to posttreatment (F3, F4, C4, P4, O2) ANT: Decreased positive delta-beta correlation from pretreatment to posttreatment (F3, F4, C3, C4, P3, P4, O1)
Harrewijn et al., 2016	HSA vs LSA participants (extreme groups)	23:0	Resting state Social judgment task Instruction Watch video Anticipation Speech Recovery	More nervous at each time point (except baseline); more avoidance after video	RS: No difference ANT: Increased negative delta-beta correlation (F3/F4/Fz) REC: Increased negative delta-beta correlation (F3/F4/Fz)
Miskovic et al., 2010	HSA vs LSA participants (extreme groups)	24 HSA (ratio unclear)	Resting state Instruction Anticipation Speech	More nervous, less confident, calm and prepared	RS: No difference ANT: Increased positive delta-beta correlation (F4)

* p-level between 0.05 and 0.1

Effect sizes are displayed when reported.

Note: SAD = social anxiety disorder; CBT = cognitive-behavioral therapy; RS = resting state; ANT = anticipation; REC = recovery; HSA = high socially anxious; LSA = low socially anxious.

Table 2

Overview of studies about early and late ERPs in face processing paradigms in social anxiety.

Author	**Participants**	**Sex ratio of target group (F:M)**	**Task**	**Stimuli**	**Behavioral results** (socially anxious relative to control)	**Early ERPs**			**Late ERPs**	
						P1	**N170**	**P2**	**P3**	**LPP**
A. Explicit tasks (and studies with both explicit and implicit instructions marked with +) - attention to emotion necessary to complete the task										
Kolassa & Miltner, 2006 +	Patients with SAD vs patients with spider phobia vs controls	9:10	Modified Stroop task (identify gender or expression)	Angry, happy, and neutral faces	No difference	P1 no diff Relation with FSS in patients with SAD	N170 ↑ angry faces, right hemisphere, during emotion identification	P2 no diff Longer latency*		
Kolassa et al., 2007 +	Patients with SAD vs patients with spider phobia vs controls	9:10	Modified Stroop task (identify colour or expression)	Schematic stimuli of angry, happy, and neutral faces	No difference	P1 ↑ overall	N170 no diff		P3 no diff	LPP no diff

Kolassa et al., 2009	Patients with SAD vs patients with spider phobia vs controls	7:8	Emotion identification task	Schematic faces that morphed into more and more intensely angry, happy or sad faces	Angry faces more arousing (also in spider phobia patients), no difference in valence ratings, emotional classifications, reaction times	P1 ↑ overall (also in spider phobia patients)	N170 no diff	P2 no diff		
Moser et al., 2008	HSA vs LSA participants (extreme groups)	15:6	Modified Eriksen Flanker task	Threatening (anger, disgust), and reassuring (happy, surprise) faces	No difference			P2 no diff threatening - reassuring faces* $\eta_p^2 = 0.08$	P3/LPP ↑ threatening target faces $\eta_p^2 = 0.12$	
Peschard et al., 2013 +	HSA vs LSA participants (extreme groups)	9:9	Modified Stroop task (identify colour or expression) Control color-	Angry, happy, and neutral faces (upright and inverted) Red, blue,	No difference in Stroop task, faster in control colour-naming task	P1 ↑ all tasks $\eta_p^2 = 0.11$	N170 no diff	P2 no diff		

			naming task	and green-coloured rectangles						
Author	**Participants**	**Sex ratio of target group (F:M)**	**Task**	**Stimuli**	**Behavioral results** (socially anxious relative to control)	**Early ERPs**			**Late ERPs**	
						P1	**N170**	**P2**	**P3**	**LPP**
Rossignol et al., 2012a	HSA vs LSA participants (extreme groups)	8:4	Emotional oddball task	Frequent stimuli: neutral faces Deviant stimuli: angry, disgusted, fearful, and happy faces	No difference	P1 ↑ $\eta_p^2 = 0.29$ all faces, emotional > neutral $\eta_p^2 = 0.12$	N170 no diff		P3b no diff	
Rossignol et al., 2007	High vs low FNE (cut off)	10:0	Identifiy deviant stimuli	Morphed faces: mix of angry and disgusted faces	Disgust detected before anger, independent of conditions (not				- N2a/P3a: earlier latencies for disgust faces. - P3b: no diff	

				Frequent stimuli: 35% angry or disgusted Deviant stimuli: 5% and 65% angry or disgusted)	in controls)					
Sewell et al., 2008	Healthy participants	12:9	Emotional oddball paradigm	Frequent stimuli: neutral faces Deviant stimuli: angry and happy faces	Not reported				Corr P3 with SA: upright > inverted faces	
Van Peer et al., 2007	High vs low BI (extreme groups)	0:20	Approach-avoidance task After cortisol and placebo administration	Angry and happy faces	More state anxiety and tension, no differences in task performance				P3 ↑ angry > happy, only in avoidant trials after cortisol administration $\eta_p^2 = 0.20$	

B. Implicit tasks - attention to emotion not necessary to complete the task										
Author	**Participants**	**Sex ratio of target group (F:M)**	**Task**	**Stimuli**	**Behavioral results** (socially anxious relative to control)	**Early ERPs**			**Late ERPs**	
						P1	**N170**	**P2**	**P3**	**LPP**
Hagemann et al., 2016	Patients with SAD vs controls	16:5	Face learning task	Angry, happy, and neutral faces	No difference Lower accuracy *	*Learning* P1 ↑ for neutral and angry faces (no effect emotion in controls) $\eta_p^2 = 0.09$ *Test* P1 no diff	*Learning* N170 no diff *Test* N170 no diff	*Learning* P2 no diff *Test* P2 no diff	*Learning* LPP ↑ for angry > neutral & happy (not in controls, not in P4) $\eta_p^2 = 0.13$ *Test* LPP ↑ for learned > novel faces (not in controls) - only for C4 electrode $\eta_p^2 = 0.07$	
Mueller et al., 2009	Patients with SAD vs controls	8:4	Modified dot-probe task	Angry-neutral, or happy-neutral face pairs	No difference (but when tested separetely SAD showed hypervigilance	P1 ↑ angry-neutral pairs $\eta_p^2 = 0.18$ ↓ probes emotionally	N170 ↓ $\eta_p^2 = 0.20$			

					to anger vs happiness, and controls not)	< neutrally cued trials $\eta_p^2 = 0.20$				
Helfinstein et al., 2008	HSA vs LSA participants (extreme groups)	12:0	Dot-probe task with neutral and social threat words as primes	Angry-neutral face pairs	Less accurate on incongruent trials than congruent trials (not in LSA)*	P1 ↑ overall		P2 ↑ overall*		
Judah et al., 2016b	HSA vs LSA participants (extreme groups)	11:9	Change detection task Cue to elicit self-focus	Disgusted and neutral faces	No difference			P2 no diff Neg corr P2 and task performance in self-focus trials (not in LSA)		
Rossignol et al., 2012b	High vs low FNE (extreme groups)	11:3	Spatial cueing task	Angry, disgusted, happy, and fearful faces	No difference	P1 ↑ overall Longer latency for probe arrow*		P2 ↑ overall	P3 no diff	

Author	Participants	Sex ratio of target group (F:M)	Task	Stimuli	Behavioral results (socially anxious relative to control)	Early ERPs			Late ERPs	
						P1	N170	P2	P3	LPP
Rossignol et al., 2013b	HSA vs LSA participants (extreme groups)	8:8	Attention-shifting paradigm with faces/bodily postures as cue	Angry, happy, and neutral faces and bodily postures of artificial humans	Overall slower response	P1 ↓ overall $\eta_p^2 = 0.13$	N170 no diff	P2 ↓ overall $\eta_p^2 = 0.13$	P3 no diff $\eta_p^2 = 0.00$	
Schmitz et al., 2012	HSA vs LSA participants (median split)	13:13	View eye gaze Report location of white dot	Neutral photos of direct or 30° left/right averted gaze	No difference	P1 ↑ averted > direct gazes* $\eta_p^2 = 0.09$	N170 no diff			LPP ↑ averted > direct gazes $\eta_p^2 = 0.13$
Rossignol et al., 2013a	High vs low FNE (extreme groups)	11:2	Spatial cueing task	Neutral-angry, neutral-happy, neutral-	No difference	P1 ↑ overall $\eta_p^2 = 0.17$ ↑ in targets replacing emotional	N170 no diff	P2 ↑ neutral-anger > neutral-fear $\eta_p^2 = 0.10$		

				disgust, and neutral-fear face pairs		face > neutral face $\eta_p^2 = 0.16$				

* p-level between 0.05 and 0.1

Effect sizes are displayed when reported.

Note: SAD = social anxiety disorder; diff = difference; FSS = Fear survey schedule; SPAI = social phobia and anxiety inventory; HSA = high socially anxious; LSA = low socially anxious; FNE = fear of negative evaluation; SA = social anxiety; BI = behavioral inhibition.

Table 3

Overview of studies about ERPs in cognitive conflict paradigms in social anxiety.

Author	**Participants**	**Sex ratio of target group (F:M)**	**Task**	**Stimuli**	**Behavioral results** (socially anxious relative to control)	**N2**	**FRN**	**ERN**	**CRN**	**Pe**
Cao et al., 2015	Patients with SAD vs controls	13:7	Island Getaway task	Neutral faces with feedback indicating social acceptance or rejection	Lower peer-acceptance expectancy in real life and in the task		FRN ↑ positive vs negative feedback $\eta_p^2 = 0.13$ Δ FRN ↑ (rejection - acceptance)			
Endrass et al., 2014	Patients with SAD vs patients with OCD vs controls	17:7	Flanker task	Arrows	No difference			ERN ↑ $\eta_p^2 = 0.16$	CRN ↑ $\eta_p^2 = 0.16$	Pe no diff $\eta_p^2 = 0.05$
Kujawa et al., 2016	Patients with SAD vs patients with GAD vs controls	13:5	Flanker task	Arrow heads	No increase in reaction time between pre and posttreatment (as			Δ ERN (error - correct response) ↑ $\eta_p^2 = 0.12$		

	Pre and posttreatment				in controls)					
Sachs et al., 2004	Patients with SAD vs controls	12:13	Standard two-tone oddball paradigm	Tones	No difference	N2 ↓				
Barker et al., 2015	HSA vs LSA participants (extreme groups)	13:12	Flanker task, alone and peer observation condition	Arrow heads	No difference			ERN ↑ peer condition vs alone, not in LSA $\eta_p^2 = 0.11$	CRN no diff	Pe no diff
Judah et al., 2016a	HSA vs LSA participants (extreme groups)	26 HSA (ratio unclear)	Trial-and-error learning task	Contour line drawings as stimuli Faces (neutral, happy, disgusted) provided performance feedback	No difference		FRN ↑ in self-focus trials before learning*	ERN ↑ $\eta_p^2 = 0.08$	CRN ↑ $\eta_p^2 = 0.08$	Pe ↑* Greater increase after learning $\eta_p^2 = 0.14$

Author	**Participants**	**Sex ratio of target group (F:M)**	**Task**	**Stimuli**	**Behavioral results** (socially anxious relative to control)	**N2**	**FRN**	**ERN**	**CRN**	**Pe**
Judah et al., 2016b	HSA vs LSA participants (extreme groups)	11:9	Change detection task Cue to elicit self-focus	Disgusted and neutral faces	No difference	N2pc for disgust faces in standard trials (LSA in self-focus trials) $\eta_p^2 = 0.16$				
Moser et al., 2008	HSA vs LSA participants (extreme groups)	15:6	Modified Eriksen Flanker task	Threatening (anger, disgust), and reassuring (happy, surprise) faces	No difference	N2 no diff			CRN no difference beteen threatening and reassuring $\eta_p^2 = 0.11$	

Rossignol et al., 2007	High vs low FNE (median split)	10:0	Identifiy deviant stimuli	Morphed faces: mix of angry and disgusted faces Frequent stimuli: 35% angry or disgusted Deviant stimuli: 5% and 65% angry or disgusted)	Disgust detected before anger, independent of conditions (not in controls)	N2b ↓ while detecting change in intensity of anger				
Van Peer et al., 2007	High vs low BI (extreme groups)	0:20	Approach-avoidance task After cortisol and placebo	Angry and happy faces	More state anxiety and tension, no differences in task performance	N2 no diff				
Van der Molen et al., 2014	Healthy participants	31:0	Social judgment paradigm	Acceptance or rejection feedback	No correlation FNE and percentage of negative judgments. Pos correlation FNE and RT for predicting acceptance and rejection		FRN no effect			

* p-level between 0.05 and 0.1

Effect sizes are displayed when reported.

Note: FRN = feedback-related negativity; ERN = error-related negativity; CRN = correct-response negativity; Pe = positive error; SAD = social anxiety disorder; OCD = obsessive-compulsive disorder; diff = difference; GAD = generalized anxiety disorder; HSA = high socially anxious; LSA = low socially anxious; FNE = fear of negative evaluation; BI = behavioral inhibition; RT = reaction time.

Table 4

Overview of studies about frontal alpha asymmetry related to social anxiety in children.

Author	**Participants**	**Sex ratio of target group (F:M)**	**Protocol**	**Results** (socially anxious relative to control)
Fox et al., 2001	Continuously inhibited, and change children At 9, 14, 24 and 48 months (based on mean scores)	4:8	Resting state	Increased right frontal activity at 9, 14, and 48* months (F3/F4)
Schmidt et al., 1999	High, middle, and low shy groups 7 years (extreme/middle groups)	4:6	Resting state Instruction Anticipation (3 parts)	Behavior: More anxious behaviors in 2nd and 3rd part of anticipation RS: No difference ANT: No difference Increased right frontal activity (F4) from 2nd to 3rd part of anticipation
Theall-Honey & Schmidt, 2006	High vs low shy children 4.5 years (extreme groups)	10:10	Resting state Watch affective videoclips (sad, anger, happy, fear) Speech	Behavior: More behavioral signs of verbal anxiety during speech RS: No difference (F3/F4), increased right central activity (C3/4) Videoclips: Increased right central activity in fear videoclip (C3/4)

Author	**Participants**	**Sex ratio of target group (F:M)**	**Protocol**	**Results** (socially anxious relative to control)
Hannesdottir et al., 2010	Healthy children at age 4.5 and 9	8:12	Age 4.5 (EEG) Resting state Cognitive control task Age 9 (HR) Resting state Instruction Anticipation Speech Recovery	Behavior: Correlations between child reported internalizing symptoms and anticipatory anxiety before speech (positive), and between anticipation anxiety and HR (positive) and HRV (negative) RS: No effect on behavior at age 9
Henderson et al., 2001	Healthy children at 9 and 48 months	51:46	Resting state	Negative reactivity predicted social wariness at age four in infant boys with right frontal activity at 9 months (F3/4)
Henderson et al., 2004	Healthy children at age 4	80-67	Resting state	Solitary-passive and reticent social play groups show increased right frontal activity (F3/4)
Fox et al., 1995	Healthy children at age 4	28:20	Resting state	No effect on inhibition/social retinence, only effect on social competence/sociability (F3/4)

* *p*-level between 0.05 and 0.1.

Effect sizes are displayed when reported.

Note: RS = resting state; ANT = anticipation; HR = heart rate, HRV = heart rate variability.

Table 5
Overview of studies about ERPs related to social anxiety in children.

Author	**Participants**	**Sex ratio of target group (F:M)**	**Task**	**Stimuli**	**Results** (socially anxious relative to control)
A. Explicit tasks - attention to emotion necessary to complete the task					
Kujawa et al., 2015	Children with anxiety disorders and healthy controls 7-19 years	All 53 (ratio unclear)	Emotional face-matching task with shape-matching trials	Angry, fearful, and happy faces	- Behavior: No difference - LPP ↑ for angry and fearful faces ($\eta_p^2 = 0.12$ and $\eta_p^2 = 0.09$)
B. Implicit tasks - attention to emotion not necessary to complete the task					
Thai et al., 2016	Community sample, children with BI were oversampled 9-12 years	50:49	Dot-probe task	Angry-neutral and neutral-neutral face pairs	- Behavior: No difference - P1, N170, N2 no effect of social anxiety - P1 to probes replacing angry faces no effect (↑ in BN) ($\eta_p^2 = 0.05$) - ↑ N2 predicted bias away from angry faces in BI

Author	**Participants**	**Sex ratio of target group (F:M)**	**Task**	**Stimuli**	**Results** (socially anxious relative to control)
C. Cognitive conflict paradigms					
Bar-Haim et al., 2003	High vs low socially withdrawn children 7-12 years (extreme groups)	11:12	Passive listening	Tones	- No behavior - P1-N1 no difference
Lackner et al., 2014	High vs low shy adolescents 12-14 years (extreme groups)	22 (ratio unclear)	Money game	Feedback indicating win or loss	- Behavior: No difference - FRN ↓
Lahat et al., 2014a	High vs low BI children 7 years (median split)	28:26	Flanker task	Fish	- Behavior: No difference - ERN ↑ ($\eta_p^2 = 0.12$) - CRN no difference - BI group was positively related to SAD symptoms, in children with relatively large ERN-CRN
Lahat et al., 2014b	High vs low BI children 7 years	40 or 41 (ratio unclear)	Flanker task	Fish	- Behavior: No difference - N2 ↑ ($\eta_p^2 = 0.09$) - Greater withdrawal and lower assertiveness in high BI

	(median split)				children with ↑ N2
McDermott et al., 2009	High vs low BI children 15 years (median split)	41 (ratio unclear)	Flanker task	Letters	- Behavior: No difference - ERN ↑ - Pe no difference - ↑ ERN related to higher risk for anxiety disorders in high BI children
Reeb-Sutherland et al., 2009	Adolescents who were high or low BI as children (latent class analysis) 13-16 years	23:20	3-stimulus auditory oddball	Tones and noises as novel stimuli	- No behavior - Novelty P3 no difference - Higher novelty P3 amplitudes = more likely to have anxiety diagnoses
Henderson, 2010	Healthy children 9-13 years	36 (ratio unclear)	Modified version of Eriksen Flanker task	Arrow heads	- Behavior: No difference - N2 no effect - Shyness predicted social anxiety in children with relatively large N2
Kessel et al., 2015	Community sample 8-10 years	175:215	Monetary reward task	Green arrow indicated win, red arrow indicated loss	- No behavior - ↑Δ FRN associated with social anxiety
Kujawa et al., 2014	Community sample 10-15 years	8:11	Island Getaway task	Feedback indicating social acceptance or rejection	- Behavior: Less rejection of co-players - ↓ (more negative) Δ FRN associated with social anxiety

Author	**Participants**	**Sex ratio of target group (F:M)**	**Task**	**Stimuli**	**Results** (socially anxious relative to control)
Lamm et al., 2014	Healthy children 7 years	58:48	Go/No-Go task	Neutral animal pictures	- Behavior: Postive relation between BI and accuracy and reaction time - Negative association between BI and N2 amplitude - Early BI was positively associated with social reticence at age 7, if N2 was increased
Tang et al., 2016	Healthy children 10 years	26:27	3-stimulus auditory oddball	Target, novel, standard tones	- Behavior: No difference - N2 no effect - P3 ↑ for target and standard tones, longer latency

Effect sizes are displayed when reported.

Note: BI = behavioral inhibition; BN = children without behavioral inhibition; CRN = correct response negativity.

Chapter 3

Putative EEG measures of social anxiety: Comparing frontal alpha asymmetry and delta-beta cross-frequency correlation

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Abstract

The goal of the current study was to examine whether frontal alpha asymmetry and delta-beta cross-frequency correlation during resting state, anticipation and recovery are EEG measures of social anxiety. For the first time, we jointly examined frontal alpha asymmetry and delta-beta correlation during resting state and during a social performance task in high (HSA) versus low socially anxious (LSA) females. Participants performed a social performance task in which they first watched and evaluated a video of a peer, and then prepared their own speech. They believed that their speech would be videotaped and evaluated by a peer. We found that HSA participants showed significant negative delta-beta correlation compared to LSA participants during anticipation of and recovery from the stressful social situation. This negative delta-beta correlation might reflect increased activity in subcortical brain regions and decreased activity in cortical brain regions. As hypothesized, no group differences in delta-beta correlation were found during resting state. This could indicate that a certain level of stress is needed to find EEG measures of social anxiety. As for frontal alpha asymmetry, we did not find any group differences. The current frontal alpha asymmetry results will be discussed in relation to the evident inconsistencies in the frontal alpha asymmetry literature. Together, our results suggest that delta-beta correlation is a putative EEG measure of social anxiety.

Introduction

Social anxiety disorder (SAD) is a common internalizing disorder that is characterized by extreme fear and avoidance of social situations (APA, 2013). Cognitive-behavioral studies have shown that patients with SAD show information-processing biases during anticipation of, and recovery from stressful social situations. For example, patients with SAD more often recall negative information about themselves during anticipation of a stressful situation, expect more negative outcomes of social situations, and show protracted post-event processing (Clark & McManus, 2002; Hirsch & Clark, 2004). A variety of studies have used social performance tasks to examine the electrophysiological correlates of these information-processing biases in response to stressful social situations (Davidson et al., 2000; Miskovic et al., 2010; Miskovic, Campbell, et al., 2011; Miskovic, Moscovitch, et al., 2011). These studies typically compare electrophysiological reactivity during baseline (resting state) with an anticipation phase in which participants are about to experience a stressful social situation. Using social performance tasks, electrophysiological investigations have shown promising results due to their high temporal precision in capturing objective brain reactivity measures during various stages of processing stressful social situations (Amodio et al., 2014; Kotchoubey, 2006; Luck, 2005). This line of work has led to two putative EEG measures of social anxiety that may aid in early detection, prevention and treatment of SAD: frontal alpha asymmetry and delta-beta cross-frequency correlation (further referred to as 'delta-beta correlation'). The goal of the current study is to validate these putative EEG measures of social anxiety by a direct comparison of frontal alpha asymmetry and delta-beta correlation in different phases of a social performance task.

An influential theoretical account proposed by Davidson (1992, 1998) claims that individuals displaying relatively stronger left frontal cortical activity are biased towards approach-related behavior, whereas individuals displaying relatively stronger right frontal cortical activity are biased towards withdrawal-related behavior (Davidson, 1992, 1998). This hemispheric lateralization of brain activity related to approach versus avoidance behavior is reflected in frontal alpha power (8-13 Hz) asymmetry metrics (Allen et al., 2004; Davidson, 1992, 1998). Frontal alpha asymmetry is typically measured by subtracting log-transformed left lateralized frontal alpha power from log-transformed right lateralized frontal alpha power (Allen et al., 2004). Since alpha power is inversely related to cortical activity, positive alpha asymmetry scores reflect relatively greater left frontal cortical activity (i.e., decreased left frontal alpha power), and negative alpha asymmetry scores reflect relatively greater right

frontal cortical activity (i.e., decreased right frontal alpha power) (Allen et al., 2004). Several reviews have shown that relatively greater right frontal cortical activity serves as a moderator for the development of various internalizing disorders, and is related to behavioral inhibition (Coan & Allen, 2003, 2004), a temperamental style relevant to the etiology of SAD (Clauss & Blackford, 2012).

Frontal alpha asymmetry research in social anxiety is limited, but there is evidence suggesting that high socially anxious (HSA), high socially withdrawn, and shy participants display relatively increased right frontal cortical activity during resting state EEG (Campbell et al., 2007; Hannesdottir, Doxie, Bell, Ollendick, & Wolfe, 2010; Schmidt, 1999). The consistency of this finding is questioned as others failed to replicate this pattern of relatively increased right frontal cortical activity during resting state in social anxiety (Beaton et al., 2008; Cole et al., 2012; Davidson et al., 2000). Findings of frontal alpha asymmetry during anticipation of stressful social situations are also mixed. Davidson et al. (2000) reported relatively elevated right frontal cortical activity in patients with SAD compared to controls, and Cole et al. (2012) showed relatively increased right frontal cortical activity in high versus low socially withdrawn participants, but only during a highly stressful condition. Namely, after viewing a video of a peer talking in an embarrassed and anxious way about a past embarrassing moment, right before participants had to prepare their own speech. However, Beaton et al. (2008) did not find differences in frontal alpha asymmetry between HSA and low socially anxious (LSA) participants when anticipating a speech task. Most studies focus on frontal alpha asymmetry during anticipation of a stressful social event, but cognitive-behavioral studies have shown that HSA participants also show information-processing biases during recovery from a stressful social event (such as post-event rumination) (Brozovich & Heimberg, 2008). Only one study focused on frontal alpha asymmetry patterns during recovery from a social stressor, but no differences were found between patients with SAD and controls (Davidson et al., 2000). Together, these studies have provided mixed evidence that frontal alpha asymmetry can be considered as an electrophysiological measure of social anxiety, either during resting state or when confronted with a social stressor.

Besides frontal alpha asymmetry, the association between delta (1-4 Hz) and beta (14-30 Hz) power (i.e. delta-beta correlation) has also been interpreted as a putative EEG measure of social anxiety. Several studies have shown that positive delta-beta correlation is increased in anxiogenic situations (for a review, see Schutter & Knyazev, 2012). It has been suggested that slow-wave oscillations in the delta frequency range stem from subcortical regions, whereas fast-wave oscillations in the beta frequency range stem from cortical regions

(Schutter & Van Honk, 2005). Significant positive delta-beta correlation has been interpreted to reflect the crosstalk between cortical and subcortical regions (Miskovic, Campbell, et al., 2011; Miskovic, Moscovitch, et al., 2011; Putman et al., 2012; Schutter & Knyazev, 2012; Schutter et al., 2006; Schutter & Van Honk, 2005; Velikova et al., 2010). Typically, significant positive delta-beta correlation is associated with anxiety. For example, significant positive delta-beta correlation is related to more attention to threat in an emotional Stroop task (Putman et al., 2012). In an anxiogenic situation, positive delta-beta correlation increases activation in a cortical network comprising the orbitofrontal and anterior cingulate cortex. An increase in delta-beta correlation is associated with an increase of delta power and connectivity in these cortical regions (Knyazev, 2011). Together, these studies revealed that anxiety in general is related to significant positive delta-beta correlation, whereas no correlation between delta and beta is related to a more relaxed state.

The possibility of delta-beta correlation as a putative EEG measure of social anxiety during resting state EEG has been demonstrated by Miskovic, Campbell, et al. (2011) and Miskovic, Moscovitch, et al. (2011). These authors reported that patients with SAD showed significant positive delta-beta correlation before cognitive behavioral therapy and no delta-beta correlation after therapy (Miskovic, Moscovitch, et al., 2011), and that children of a parent with SAD showed significant positive delta-beta correlation compared to typically developing children (Miskovic, Campbell, et al., 2011). Obviously, these results should be interpreted with caution because these studies only report on resting state data, while others have shown that delta-beta correlation is increased only in an anxious state (Schutter & Knyazev, 2012). Moreover, the latter study was based on a small sample size (n = 6). However, delta-beta correlation does seem to be relevant in the pathophysiology of social anxiety, as significant positive delta-beta correlation has been found in HSA compared to LSA during anticipation of giving a speech in front of a camera (Miskovic et al., 2010). This significant positive delta-beta correlation was associated with higher levels of self-reported nervousness, less confidence, less calmness, less preparedness, and poorer estimates of the anticipated speech performance (Miskovic et al., 2010). Thus, although few studies have investigated delta-beta correlation during resting state and anticipation, there is preliminary evidence suggesting that significant positive delta-beta correlation during resting state and anticipation could be an EEG measure of social anxiety (Miskovic et al., 2010; Miskovic, Campbell, et al., 2011; Miskovic, Moscovitch, et al., 2011).

Due to the mixed results about these putative measures in prior investigations, the main objective of this study was to validate whether frontal alpha asymmetry and delta-beta

correlation can be considered as electrophysiological measures of social anxiety. Moreover, we included three phases of examination for each participant (i.e., resting state, anticipation, and recovery), which allowed for determining whether group differences in frontal alpha asymmetry and delta-beta correlation can be detected as trait (i.e., during resting state) or as state (i.e., during anticipation of and/or recovery from a social stressor) phenomena. Notably, electrophysiological studies have focused mostly on anticipation and have shown inconsistent results, thus the current inclusion of a recovery phase could yield a better understanding of the state vs. trait characteristics of these alleged EEG measures, as well as their temporal relevance in biased information processing in social anxiety (i.e., biased anticipatory attention vs ruminative thinking during recovery from a social stressor). Similar to previous EEG studies, we used a social performance task to elicit arousal associated with social performance anxiety (Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Westenberg et al., 2009). In our version of the social performance task, HSA and LSA participants watched and evaluated a video of a peer before preparing their own speech that would be videotaped and evaluated by a peer. Unique about this study is that we focus on both frontal alpha asymmetry and delta-beta correlation – measured in the same participants – during three phases of the experiment. We hypothesized that if right frontal cortical activity and positive delta-beta correlation are EEG measures of social anxiety, these measures would be increased in HSA compared to LSA participants. Although results from previous studies are inconsistent, we used the theoretical background to hypothesize in which phase these putative EEG measures would be present. First, since increased right frontal cortical activity (as measured with frontal alpha asymmetry) is related to avoidance-related behavior (Davidson, 1992, 1998), we hypothesized that this would be present only during anticipation, not during resting state or recovery, because of mixed findings in resting state studies and because avoidance is not possible after the stressful social event. Second, since significant positive delta-beta correlation is related to crosstalk between cortical and subcortical regions in an anxious state (Schutter & Van Honk, 2005), we hypothesized that this could be present during both anticipation and recovery, and not during resting state.

Methods

Participants

Participants were selected from 386 female undergraduate students who completed self-reports of the Liebowitz Social Anxiety Scale (LSAS) (Liebowitz, 1987). As studies about

gender differences in frontal alpha asymmetry have shown inconsistent results (Jesulola et al., 2015), we included only female participants to reduce interindividual variability. Based on their LSAS scores, participants were assigned to either a LSA group (LSAS score < 30) or HSA group (LSAS score ≥ 60). Cutoff scores were based on results from Mennin et al. (2002) indicating that LSAS scores lower than 30 are not associated with clinical social anxiety, whereas LSAS scores of 60 and higher are associated with generalized SAD. We administered the LSAS first as screening, and again after the experiment to validate that participants were still high or low socially anxious during the experiment. We excluded participants who showed an extreme difference (greater than 2 SD) in LSAS score between screening and testing. The correlation between LSAS score at screening and during testing was high ($\tau = 0.66$, $p < 0.001$). Three HSA participants were excluded due to data acquisition problems ($n = 1$), extreme difference between LSAS scores during screening and testing ($n = 1$), and unwillingness to participate in the social performance task ($n = 1$). Two LSA participants were excluded due to extreme difference between LSAS scores ($n = 1$) and missing questionnaire data ($n = 1$). This resulted in a final sample of 23 HSA (mean age 19.56 years, $SD = 1.43$) and 33 LSA (mean age 19.81, $SD = 1.45$) female participants. Age did not differ between the groups, $F(1, 54) = 0.41$, $p = 0.53$, $\eta^2 = 0.01$.

All participants were healthy, free from psychoactive medication, right-handed, as confirmed with the Edinburgh Handedness Inventory (Oldfield, 1971), and had normal or corrected-to-normal vision. Participants were recruited from or within the proximity of Leiden University, and were rewarded with €17 or course credit for their participation. All participants provided signed informed consent. This procedure is according to the Declaration of Helsinki. The ethical committee of the Institute of Psychology of Leiden University reviewed and approved this study.

Procedure

Participants first received an explanation about the EEG procedure and signed the informed consent form. After attaching the electrodes, the EEG protocol started with measuring EEG resting state for 5 minutes (eyes closed). Thereafter participants performed a social judgment task (data reported elsewhere) and the social performance task. Finally, participants filled out the questionnaires and were debriefed. The experiment took 2.5 hours in total.

Social Performance Task

To measure EEG activity during a stressful social situation, we used a modified version of the social performance task presented in Rinck et al. (2013). Our social performance task comprised five phases (instruction, video, anticipation, speech, recovery), which were presented in a fixed order and are depicted in Figure 1. First, participants were informed about the task, because they did not know beforehand about this task. This was done to avoid anticipatory stress during resting state EEG that was collected prior to the social performance task. Participants were explained that they would view and judge a video of a peer telling about her positive and negative characteristics (rating 1, see Supplementary data 1 for these results). Then, participants prepared a speech about their own positive and negative characteristics (anticipation). Using a cover story it was explained that their video would be shown to a peer, and that this peer would judge the participant's video (this was not the case). Participants were asked how they thought their video would be judged by a peer (rating 2, see Supplementary data 1 for these results). After participants gave their speech for three minutes in front of a camera, they had five minutes to relax (recovery). All participants reported after the experiment that they believed the cover story.

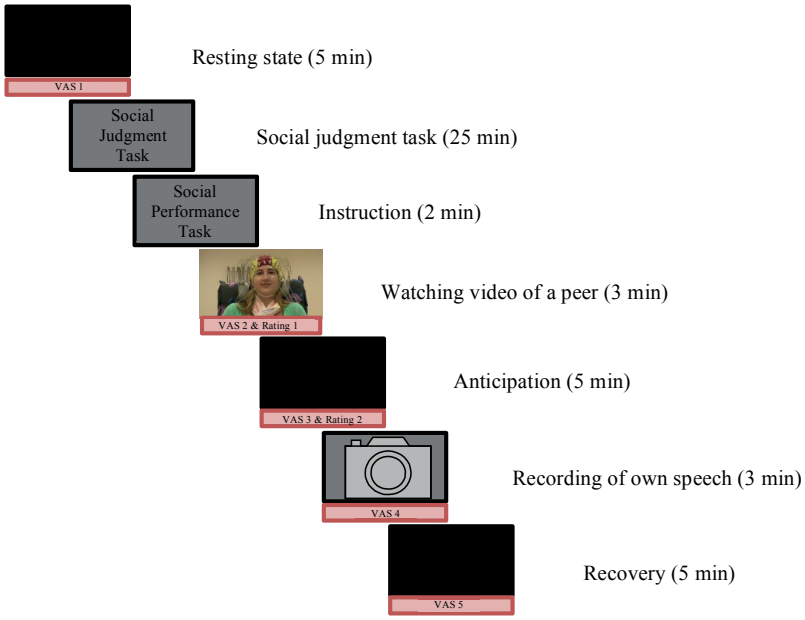


Figure 1. Overview of the experiment. EEG was recorded during resting state, anticipation, and recovery. Results of the social judgment task will be reported elsewhere.

Task-related nervousness and avoidance. At five time points during the social performance task (see Figure 1), we asked participants to indicate how nervous they felt on a visual analogue scale (VAS) from 0 (not at all) to 100 (very much), and how much they felt like doing the next part of the experiment on a VAS from 0 (not at all) to 100 (very much). The 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 a situation and do nothing about it.

Self-report questionnaires

Social anxiety was measured with the LSAS (Liebowitz, 1987), which consists of 24 social situations. Participants rated on a 4-point Likert scale their anxiety (0 = none, 3 = severe) and avoidance (0 = never, 3 = usually) in each of these situations in the last week. The LSAS has a high internal consistency ($\alpha > 0.90$) (Liebowitz, 1987). In addition, to validate the HSA and LSA groups based on social anxiety related constructs, we furthermore administered questionnaires that indexed fear of negative evaluation (Bögels & Reith, 1999), fear of

positive evaluation (Weeks, Heimberg, & Rodebaugh, 2008), post-task rumination (Edwards, Rapee, & Franklin, 2003; Miers, Blote, Heyne, & Westenberg, 2014), and depression (Beck et al., 1996).

EEG recording and signal processing

EEG was measured with 64 Ag-AgCl electrodes mounted in an elastic electrode cap (10/20 placement) using the BioSemi Active Two system (Biosemi, Amsterdam, The Netherlands). EEG was recorded with a sampling rate of 1024 Hz. The common mode sense (CMS) and driven right leg (DRL) replaced the conventional ground electrode, and CMS was used as online reference. Vertical ocular movements were measured with electrodes placed above and below the left eye. Horizontal ocular movements were measured with electrodes placed at left and right canthus. Two electrodes were placed at left and right mastoid for offline referencing. We additionally measured the electrocardiogram via the modified lead-2 placement, but these data will be reported elsewhere.

EEG time series were analyzed with BrainVision Analyzer (BVA; Brain Products GmbH). EEG-channels were re-referenced to the average of all EEG electrodes[3], and filtered between 0.1-50 Hz (24 dB/oct), with a 50 Hz notch filter. Epochs of 4 sec (4096 samples) were created 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 BVA. 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 whole segment was removed during both manual and automatic artifact rejection. HSA and LSA participants did not differ in their number of clean epochs per phase of the task (resting state, anticipation, and recovery), all *p*s > 0.05 (see Table 1).

[3] An average reference scheme was employed (1) to allow for a better comparison of the current data with results previously obtained in HSA samples (Cole et al., 2012; Davidson et al., 2000), and (2) because the average reference scheme best approximates an inactive reference provided a large array of electrodes is used (Allen et al., 2004). Furthermore, Allen et al. (2004) demonstrated that beta power shows a negative relation to brain activity when a linked ears reference was used. Results of the current study remained the same when we used an average of the mastoids as reference scheme.

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)[4], alpha (8-13 Hz), total beta (14-30 Hz), low beta (14-20 Hz), and high beta (20-30 Hz) frequency bands. With respect to beta power, we distinguished between high and low beta power to examine the contribution of high and low beta separately to the delta-beta correlation results, and to allow for a better comparison of our study with those using only high or low beta bands (Miskovic et al., 2010; Miskovic, Moscovitch, et al., 2011).

We focused on relative power values, because relative power better reflects cortical activity (Allen et al., 2004; Cook, O'Hara, Uijtdehaage, Mandelkern, & Leuchter, 1998) and is less confounded by scalp thickness and electrical resistance compared to absolute power (Allen et al., 2004; Knyazev, Savostyanov, & Levin, 2004). The use of relative power decreases data variability and increases the probability of finding relationships between EEG and personality (Knyazev, 2007; Knyazev et al., 2004). Power estimates from all spectral bands were averaged across trials and log-transformed to obtain normal distributions.

Table 1

Mean, standard deviation (SD), and range of the number of clean epochs per phase in the social performance task (resting state, anticipation, and recovery), per group.

		Mean	SD	Minimum	Maximum
Resting state	HSA	88.83	8.70	68	99
	LSA	91.64	5.04	79	99
Anticipation	HSA	87.13	12.05	49	98
	LSA	86.97	8.87	60	98
Recovery	HSA	90.30	10.55	58	99
	LSA	91.09	9.07	54	99

Frontal alpha asymmetry. As previous studies have led to inconsistent results with regard to the localization of peak alpha asymmetry scores, we computed composite alpha scores based on the average of alpha power in a left (F3, F5) and right (F4, F6) frontal cluster.

[4] We examined whether the delta power measure was confounded by arterial activity, by running an independent component analyses for a random selection of ten participants, and extracting the ECG component from the EEG data. Results showed that this ECG corrected delta measure did not differ from the uncorrected delta power measure (for both relative and absolute power during resting state, for electrodes F3, F4 and Fz, all *p*s > 0.05) and thus delta power uncorrected for ECG activity is reported here.

Moreover, this approach avoids an arbitrary choice of electrode pairs. Alpha asymmetry values were obtained within participants by subtracting left frontal alpha power from right frontal alpha power (ln[right] – ln[left]), which corrects for overall alpha power levels and reduces individual differences related to skull thickness (Allen et al., 2004).

Delta-beta correlation. Power values for electrodes F3, Fz, F4 were averaged into composite frontal delta and beta power values (Putman, 2011; Putman et al., 2012). Kendall's tau correlations were computed between delta and beta power separately for each group (HSA and LSA) in each condition (resting state, anticipation, recovery) for total beta, low beta, and high beta power separately. We used this between-subjects measure of delta-beta correlation to directly compare our findings with previous studies in social anxiety (Miskovic et al., 2010; Miskovic, Campbell, et al., 2011; Miskovic, Moscovitch, et al., 2011). In addition, to be able to consider individual differences, we computed a within-subjects measure of delta-beta correlation (see Supplementary data 2).

Statistical analysis

The analyses were performed in three steps to examine (1) self-report data, (2) frontal alpha asymmetry, and (3) delta-beta correlation. First, we analyzed self-report data to validate the groups and the social performance task. Group differences in the questionnaires were examined using ANOVAs, and Mann-Whitney tests for variables that were not normally distributed. We examined group differences in nervousness and avoidance during the social performance task, using non-parametric Mann-Whitney tests per time point, since these variables were not normally distributed. We applied a Bonferroni correction ($\alpha = 0.01$) to correct for multiple comparisons. Second, we analyzed frontal alpha asymmetry during (a) resting state, and (b) the social performance task, using non-parametric Mann-Whitney tests, since the log-transformed alpha asymmetry scores were not normally distributed. Third, we analyzed delta-beta correlation during (a) resting state, and (b) the social performance task. We examined differences between groups in Kendall's tau correlation coefficients using a Fisher's r-to-Z transformation for resting state and social performance task separately. For all analyses, we used IBM SPSS Statistics 21 (IBM Corporation, 2012) and set alpha at 0.05. Greenhouse-Geisser corrections were used whenever appropriate, but uncorrected degrees of freedom were reported for transparency.

Results

Behavioral data

Self-report questionnaires. Table 2 shows the means and standard deviations of HSA and LSA participants on the questionnaires. Compared to LSA participants, HSA participants displayed significant higher levels of social anxiety (during screening and after the experiment), depression, fear of negative evaluation, fear of positive evaluation, and negative rumination (all *p*s < 0.001).

Table 2

Overview of mean (SD) social anxiety, depression, fear of negative evaluation, fear of positive evaluation and rumination scores in HSA and LSA participants.

	HSA (n = 23)	LSA (n = 33)	U	z	p	r
LSAS (screening)	73.13 (10.98)	19.18 (7.72)	759.00	6.33	<0.001	0.42
LSAS (testing)	75.35 (18.87)	25.33 (12.19)	756.50	6.28	<0.001	0.42
Depression (BDI)	12.22 (6.71)	6.45 (4.78)	590.00	3.52	<0.001	0.24
Rumination (positive)	7.04 (4.47)	8.67 (5.69)	333.00	-0.78	0.44	-0.05
			F		p	η^2
Fear of negative evaluation (FNE)	31.61 (8.66)	18.76 (11.40)	20.81		<0.001	0.28
Fear of positive evaluation (FPES)	37.83 (10.85)	21.18 (13.63)	23.77		<0.001	0.31
Rumination (negative)	27.57 (9.27)	13.27 (9.04)	33.16		<0.001	0.38

Nervousness. We compared nervousness between HSA and LSA participants at five time points during the social performance task (Figure 2a). After Bonferroni correction (α = 0.01), there was no difference between HSA and LSA participants at baseline, U = 529.00, z = 2.50, p = 0.012, r = 0.15. HSA were more nervous than LSA participants at all the other time points, respectively U = 576.00, z = 3.27, p = 0.001, r = 0.20, U = 540.00, z = 2.67, p = 0.008, r = 0.16, U = 560.50, z = 3.02, p = 0.003, r = 0.18, U = 575.50, z = 3.27, p = 0.001, r = 0.20.

Avoidance. After Bonferroni correction (α = 0.01), there was no difference between HSA and LSA participants at baseline, U = 281.50, z = -1.63, p = 0.102, r = -0.10. After the video, HSA participants felt less like doing the task than LSA participants, U = 225.50, z = -

2.57, $p = 0.010$, $r = -0.15$. During the rest of the task, there was no difference between HSA and LSA participants, respectively $U = 237.50$, $z = -2.37$, $p = 0.02$, $r = -0.14$, $U = 319.00$, $z = -1.01$, $p = 0.31$, $r = -0.06$, $U = 289.00$, $z = -1.51$, $p = 0.13$, $r = -0.09$.[5]

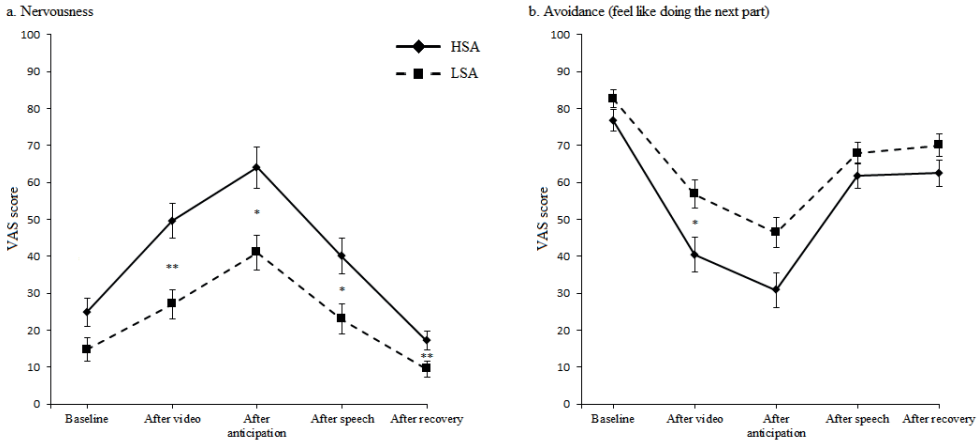


Figure 2. Nervousness (a) and avoidance (b) during the social performance task. HSA participants showed more nervousness during the social performance task and avoidance after the video compared to LSA participants.

Note: ** $p < 0.002$ * $p < 0.01$, error bars represent standard error.

Frontal alpha asymmetry

Resting state. As hypothesized, there was no difference between HSA and LSA participants in frontal alpha asymmetry during resting state, $U = 289.00$, $z = -1.51$, $p = 0.13$, $r = -0.12$ (Figure 3).

Social performance task. HSA and LSA participants did not differ in frontal alpha asymmetry during anticipation and recovery, respectively $U = 334.00$, $z = -0.76$, $p = 0.45$, $r = -0.06$, $U = 359.00$, $z = -0.34$, $p = 0.73$, $r = -0.03$ (Figure 4).

[5] There were no correlations between the EEG measures (alpha asymmetry, delta, beta, low beta, and high beta power during resting state, anticipation and recovery), task-related nervousness and avoidance, and questionnaires after correction for multiple comparisons (240 correlations, $\alpha = 0.0002$).

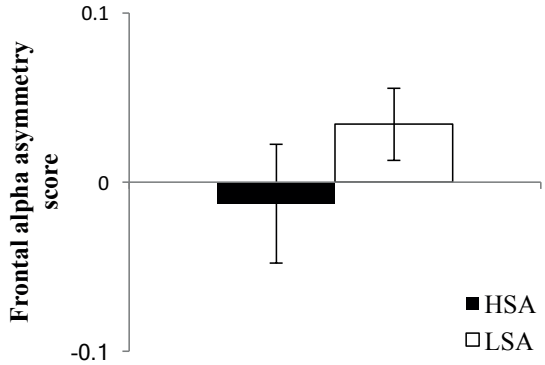


Figure 3. Frontal alpha asymmetry scores for HSA and LSA participants during resting state. Note: Error bars represent standard error.

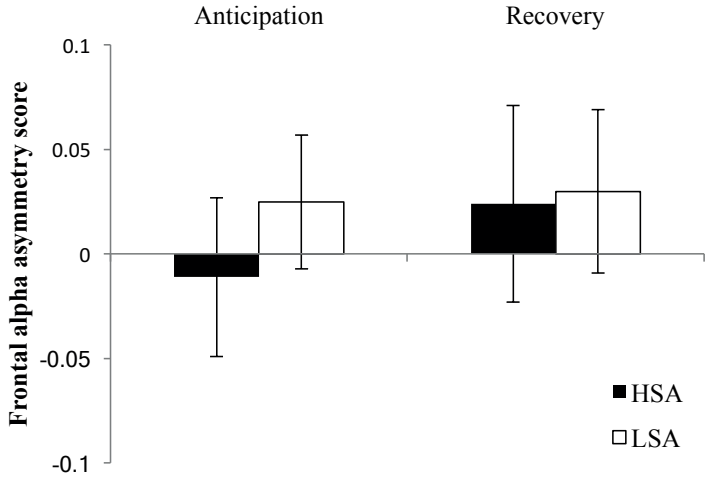


Figure 4. Frontal alpha symmetry scores for HSA and LSA participants during anticipation and recovery (error bars represent standard error).

Delta-beta cross-frequency correlation

Table 3 shows the absolute and relative delta and beta power across the groups and phases of the social performance task.

Table 3

Absolute and relative delta and beta power (log transformed) for the frontal cluster in HSA and LSA participants during resting state (RS), anticipation (ANT), and recovery (REC).

		HSA		LSA	
		Absolute	Relative	Absolute	Relative
RS	Delta (1-4 Hz)	-0.67 (0.08)	0.55 (0.05)	-0.57 (0.06)	0.46 (0.07)
	Beta (14-30 Hz)	-2.93 (0.12)	-1.69 (0.06)	-2.91 (0.10)	-1.86 (0.06)
	Low beta (14-20 Hz)	-2.57 (0.13)	-1.34 (0.08)	-2.56 (0.11)	-1.52 (0.06)
	High beta (20-30 Hz)	-3.24 (0.11)	-2.00 (0.05)	-3.22 (0.09)	-2.16 (0.07)
ANT	Delta (1-4 Hz)	-0.63 (0.09)	0.70 (0.04)	-0.53 (0.07)	0.66 (0.04)
	Beta (14-30 Hz)	-2.64 (0.13)	-1.28 (0.06)	-2.54 (0.11)	-1.33 (0.05)
	Low beta (14-20 Hz)	-2.45 (0.13)	-1.09 (0.06)	-2.37 (0.11)	-1.16 (0.04)
	High beta (20-30 Hz)	-2.81 (0.13)	-1.46 (0.08)	-2.68 (0.12)	-1.47 (0.07)
REC	Delta (1-4 Hz)	-0.84 (0.08)	0.64 (0.05)	-0.86 (0.07)	0.61 (0.04)
	Beta (14-30 Hz)	-2.76 (0.13)	-1.09 (0.08)	-2.92 (0.11)	-1.19 (0.06)
	Low beta (14-20 Hz)	-2.62 (0.12)	-1.12 (0.06)	-2.75 (0.10)	-1.26 (0.04)
	High beta (20-30 Hz)	-2.90 (0.14)	-1.40 (0.09)	-3.06 (0.11)	-1.57 (0.07)

Resting state. As hypothesized, delta-beta correlation did not differ between HSA and LSA participants during resting state (HSA: τ = -0.01, LSA: τ = 0.23, Z = 0.87, p = 0.38) (Figure 5). Similar results were found for the within-subjects measure of delta-beta correlation (see Supplementary data 2).

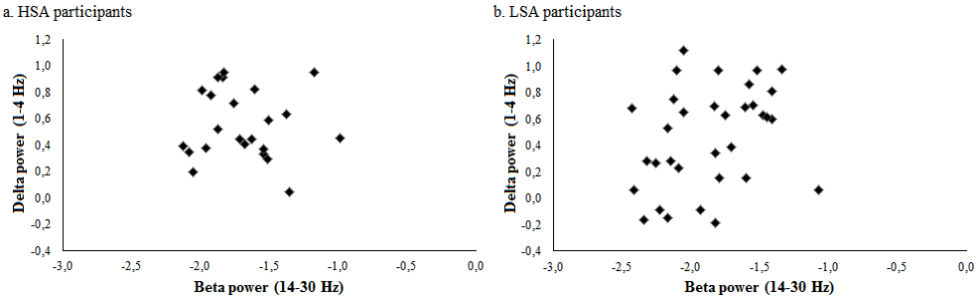


Figure 5. Scatterplots of relative total beta and relative delta power in HSA and LSA participants during resting state.

Social performance task. First, we examined group differences in delta-beta correlation during anticipation and recovery (Table 3). During the anticipation phase, delta-beta correlation was significantly more negative in HSA (τ = -0.76) than LSA (τ = -0.39) participants, Z = 2.07, p = 0.04. During the recovery phase, delta-beta correlation was also significantly more negative in HSA (τ = -0.61) than in LSA (τ = -0.13) participants, Z = 1.98, p = 0.05 (Figure 6). However, when we used a Bonferroni correction, these findings were not significant. Notably, similar analyses performed on the within-subjects measure of delta-beta correlation yielded a significantly more negative correlation during the anticipation phase, relative to the recovery phase, in both groups (see Supplementary data 2).

To examine more closely whether the above findings were driven by either low or high beta frequencies, the analyses were run for low (14-20 Hz) and high (20-30 Hz) beta power separately. As shown in Table 4, cross-frequency correlations between delta and low or high beta did not differ between HSA and LSA participants.

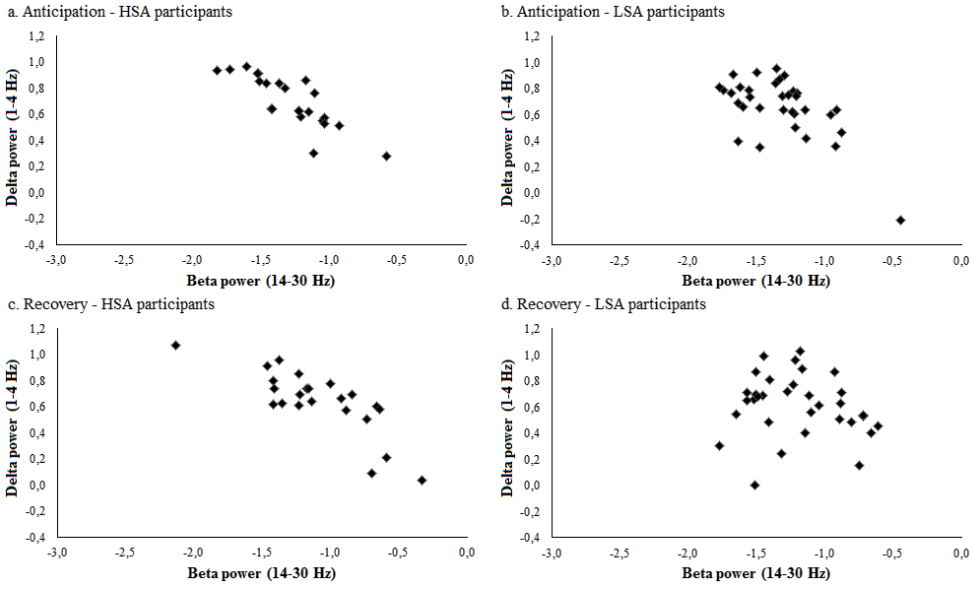


Figure 6. Scatterplots of relative total beta and relative delta power in HSA and LSA participants during the social performance task.

Table 4

Correlations between delta and beta (also separately for low and high beta) per condition per group.

		τ		*Z*	*p*
		HSA	LSA		
Anticipation	Total beta	-0.76	-0.39	2.07	0.04
	Low beta	-0.44	-0.34	0.42	0.67
	High beta	-0.67	-0.31	1.67	0.10
Recovery	Total beta	-0.61	-0.13	1.98	0.05
	Low beta	-0.33	-0.15	0.65	0.52
	High beta	-0.48	-0.004	1.79	0.07

* Significant after Bonferroni correction (α = 0.008)

Discussion

The goal of the current study was to validate whether frontal alpha asymmetry and delta-beta correlation during resting state and a social performance task are putative electrophysiological measures of social anxiety. We used a social performance task to measure EEG activity during anticipation of, and recovery from a stressful social situation. At the behavioral level, results showed that HSA participants were more nervous during the social performance task and felt less like doing the anticipation than LSA participants. For frontal alpha asymmetry, no significant differences were found between HSA and LSA participants during resting state, anticipation, and recovery. Delta-beta correlation differed between HSA and LSA participants. That is, HSA participants displayed enhanced negative delta-beta correlation compared to LSA participants during anticipation of, and recovery from a social performance task. This study is the first to directly compare frontal alpha asymmetry and delta-beta correlation, and shows that delta-beta correlation is a putative EEG measure of social anxiety.

During resting state, frontal alpha asymmetry did not differ between HSA and LSA participants in our study, which is in line with our hypothesis and with previous results in patients with SAD versus controls (Davidson et al., 2000), HSA versus LSA participants (Beaton et al., 2008), and high versus low socially withdrawn participants (Cole et al., 2012). Several other findings contradict these results. For example, Moscovitch et al. (2011) found that patients with SAD showed relatively more left frontal cortical activity after cognitive-behavioral treatment. Although, it was not tested whether these patients showed relatively

increased right frontal cortical activity before treatment. Further, shyness was related to frontal alpha asymmetry during resting state (Beaton et al., 2008; Schmidt, 1999). This relation with frontal alpha asymmetry was only found for shyness, not for social anxiety (Beaton et al., 2008). As shyness might be part of a social anxiety disorder spectrum (Stein et al., 2004), we expected the same results in these groups as in HSA participants. This might indicate that shyness and social anxiety are separate constructs: shyness is a more general discomfort in novel social situations (Henderson, 2010), whereas social anxiety is related to fear of negative evaluation (APA, 2013). If this is indeed the case, the effects of shyness cannot be generalized to social anxiety. Based on the current findings and prior inconsistencies in the frontal alpha asymmetry literature, we suggest that frontal alpha asymmetry is possibly not a stable EEG measure of social anxiety.

During the anticipation and recovery phases of the social performance task, frontal alpha asymmetry did not differ between HSA and LSA participants. With regard to the anticipation phase, our current results corroborate previous findings. That is, Beaton et al. (2008) demonstrated that frontal alpha asymmetry did not differ between HSA versus LSA participants during a speech preparation task. Further, Cole et al. (2012) have shown that high socially withdrawn individuals did not differ in frontal alpha asymmetry from low socially withdrawn individuals when they watched a benign movie. However, after watching an anxious video, a significant increase in right frontal cortical activity was observed in high socially withdrawn participants. Probably, our results on anticipatory activity in the social performance task can best be compared with the benign condition in the Cole et al. (2012) study, because our confederate did not talk in an embarrassed and anxious way (see Supplementary data 1). Our current findings are in contrast with Davidson et al. (2000) who showed that patients with SAD could be characterized by relatively increased right frontal cortical activity compared to controls when anticipating a public speech. These contradicting findings could possibly be related to the degree of anxiety elicited by the task. That is, our social performance task elicited a considerable level of nervousness in both HSA and LSA participants, while the social performance task used in the study of Davidson et al. (2000) only elicited feelings of anxiety in patients with SAD. Thus, in contrast to the Davidson et al. (2000) study, our social performance task elicited higher levels of psychological arousal in the control sample, which may have resulted in deflated group differences at the electrophysiological level. During recovery, Davidson et al. (2000) showed no differences between patients with SAD and controls, which was confirmed by our results. In conclusion,

it remains unclear whether frontal alpha asymmetry during a social performance task is related to social anxiety.

These mixed results in previous studies on frontal alpha asymmetry as a putative EEG measure of social anxiety could be due to the alleged influence of depression on alpha asymmetry levels. Indeed, Thibodeau et al. (2006) hypothesized that frontal alpha asymmetry in anxiety might be explained by comorbid depression, because effect sizes of frontal alpha asymmetry studies were near zero in samples that included anxious participants without comorbid depression. Unfortunately, most studies on frontal alpha asymmetry in social anxiety do not report comorbid depression. Future studies should compare HSA participants with high and low comorbid depression to disentangle the effects of social anxiety and depression on frontal alpha asymmetry.

The second putative EEG measure we examined here was delta-beta correlation. As hypothesized, delta-beta correlation did not differ between HSA and LSA participants during resting state. Indeed, delta-beta correlation is generally only increased in response to anxiogenic situations (Knyazev, 2011; Knyazev et al., 2006), and not during resting state in HSA and LSA participants (Miskovic et al., 2010). Although, children with a parent with SAD showed more delta-beta correlation during resting state compared to children with healthy parents (Miskovic, Campbell, et al., 2011). Furthermore, patients with SAD showed significant delta-beta correlation before cognitive behavioral treatment (Miskovic, Moscovitch, et al., 2011). Taken together, there is mixed evidence that delta-beta correlation during resting state is an EEG measure of social anxiety.

During the anticipation phase of the social performance task, we found enhanced negative delta-beta correlation in HSA compared to LSA participants. This is in line with findings of Miskovic et al. (2010) and Miskovic, Moscovitch, et al. (2011) who also found a difference between respectively HSA and LSA participants, and patients with SAD and LSA participants. They found increased positive delta-beta correlation that was accompanied by more nervousness, less confidence, less calmness, les preparedness, and poorer estimates of the anticipated speech performance (Miskovic et al., 2010). Interestingly, LSA participants also showed increased negative delta-beta correlation during anticipation compared to resting state in our study. This could be related to increased nervousness, but this should be confirmed by future research. During recovery, we found enhanced negative delta-beta correlation in HSA compared to LSA participants. It seemed like HSA participants showed a prolonged reaction to the social performance paradigm, whereas the reaction of LSA participants went back to baseline. However, these findings should be interpreted with caution

since our sample size was modest, the findings were not significant after Bonferroni correction, and delta-beta correlation was a between-subjects measure, so we cannot draw any conclusions on the within-subjects level. There were no previous studies in social anxiety that have measured delta-beta correlation during recovery. To summarize, this and previous studies have shown that delta-beta correlation during anticipation and recovery are putative EEG measures of social anxiety, but this should be confirmed by future research.

We have found negative delta-beta correlations, whereas previous studies have found positive delta-beta correlations (Miskovic et al., 2010; Miskovic, Campbell, et al., 2011; Miskovic, Moscovitch, et al., 2011). Typically, delta-beta correlation is interpreted as reflecting the crosstalk between cortical and subcortical brain regions (Schutter & Knyazev, 2012), where significant positive delta-beta correlation would indicate stronger functional coherence between cortical and sub-cortical regions (Putman, 2011). Non-significant delta-beta correlation is interpreted as the absence of functional coherence between cortical and subcortical regions (Miskovic & Schmidt, 2009; Schutter & Knyazev, 2012). Following this line of reasoning, significant negative delta-beta correlation would also indicate stronger functional coherence, only in a different direction. General models of anxiety suggest that increased activity in the amygdala and decreased activity in the prefrontal cortex bias the brain towards threat-related responses (Bishop, 2007). This inverse relationship between cortical and subcortical brain regions would suggest a negative correlation between oscillations that stem from these regions, as found in our study.

The positive delta-beta correlation observed in previous studies could be due to differences in the power measures calculated. Notably, previous studies have not specified which EEG power measure (i.e., absolute or relative) has been used. In the current study, we calculated relative EEG power as it better reflects cortical activity and is less confounded by scalp thickness and electrical resistance (Allen et al., 2004; Cook et al., 1998; Knyazev, 2007; Knyazev et al., 2004). It should be noted that when analyzing the delta-beta correlation using absolute power, we did obtain positive correlations, however, no differences between groups were found. Thus the robustness of our current findings using relative power should be tested in future studies. Another possible reason for the unexpected negative delta-beta correlation could be that the relationship between delta-beta correlation and stress is not linear but U-shaped. It is possible that at a certain level of stress the relation between delta and beta power changes. Our social performance paradigm might be more stressful, because LSA participants also showed increased nervousness during the task. As a result, our study might have passed the threshold which resulted in negative delta-beta correlation. Future research with different,

increasingly stressful phases should give more insight in the relation between delta-beta correlation and stress.

Despite the strength of comparing frontal alpha asymmetry and delta-beta correlation during resting state, anticipation, and recovery in the same sample, this study has a few limitations that should be taken into account. First, we tested a modest sample which consisted only of female participants, which limits generalization of the current findings. Second, delta-beta correlation has been computed as a between-subjects measure, to compare the findings with previous studies using the same measure (Miskovic et al., 2010; Miskovic, Campbell, et al., 2011; Moscovitch et al., 2011). However, this warrants caution with interpretation of delta and beta power within participants. When we analyzed the data in a within-subjects way (see Supplementary data 2), the data showed the same pattern, but the differences between HSA and LSA participants were not significant. Knyazev (2011) has compared between-subjects and within-subjects measures of delta-beta correlation, and concluded that these measures were similar. However, the between-subjects analysis revealed more significant results than the within-subjects analysis (Knyazev, 2011). This could be related to a difference in power between the two types of analysis, which could also explain the differences in the current study. Third, EEG during resting state was measured with eyes closed, whereas EEG during anticipation and recovery was measured with eyes open. This allowed us to compare our findings with existing studies on social anxiety – that reported results on eyes-opened task data. A potential drawback is that it interfered with direct comparison of EEG resting state data and data of the social performance task within this study. Indeed, it has been shown that EEG oscillations differ between eyes-open and eyes-closed resting state conditions (Barry, Clarke, Johnstone, Magee, & Rushby, 2007). Future studies should measure resting state, anticipation and recovery in the same way, to be able to directly compare EEG oscillatory power during these phases. Fourth, social anxiety has a high comorbidity with other anxiety disorders, such as depression and substance abuse disorders (Rapee & Spence, 2004). As previously noted, comorbid depression could influence the relation between social anxiety and frontal alpha asymmetry. Besides obtaining reliable electrophysiological measures of social anxiety, future research should preferably focus on the specificity of such measures for social anxiety. Such specificity may have important consequences for characterization of biomarkers, as well as the development of treatment procedures of SAD.

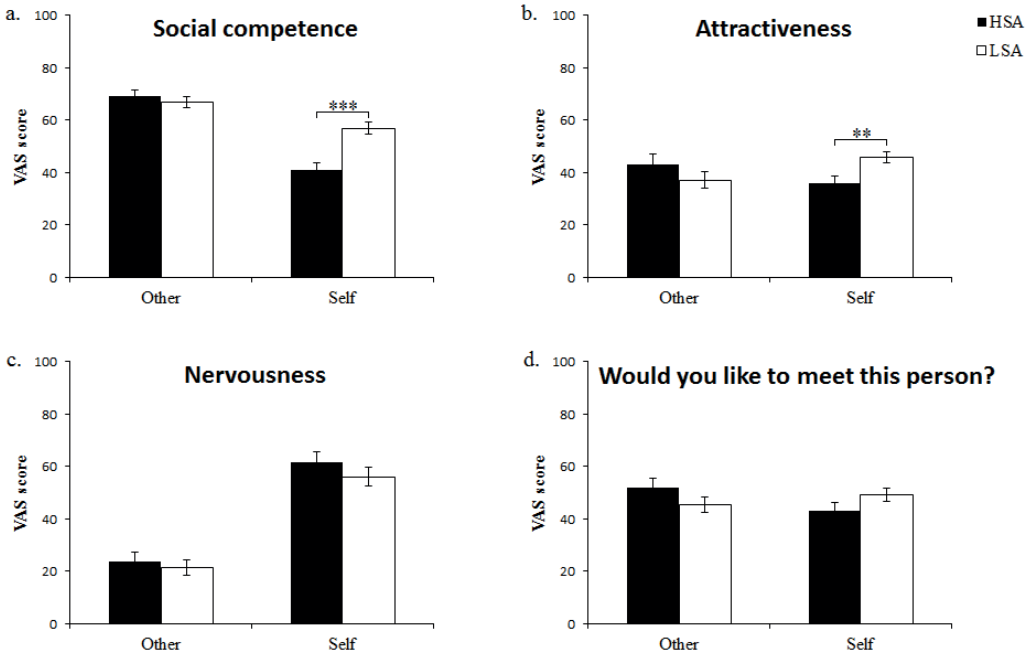
In conclusion, the current study provided a detailed characterization of frontal alpha asymmetry and delta-beta correlation as putative EEG measures during resting state and a

social performance task in HSA and LSA participants. Our results suggest that delta-beta correlation during anticipation of and recovery from a social performance task (i.e., giving a speech in front of a camera) is a putative electrophysiological measure of social anxiety. Moreover, by including both resting state, as well as task-related EEG data we were able to demonstrate that a certain level of stress might be needed to find EEG measures of social anxiety.

Supplementary data 1

Ratings of the video and expectations of own speech

After participants viewed the video of a peer, they indicated how socially competent, attractive and nervous the person on the video was, and whether they would like to meet the person. Right before participants had to give their own speech, we asked them to indicate how they expected to be judged by another person on the same four questions (see Supplementary figure 1). For social competence, all participants judged the other as more socially competent than they expect to be judged themselves, $F(1, 54) = 62.45$, $p < 0.001$, *partial* $\eta^2 = 0.54$, and HSA participants showed overall lower ratings, $F(1, 54) = 9.04$, $p = 0.004$, *partial* $\eta^2 = 0.14$. Furthermore, there was a significant interaction effect between group and ratings, $F(1, 54) = 14.26$, $p < 0.001$, *partial* $\eta^2 = 0.21$, indicating that HSA participants expected to be judged as less socially competent than LSA participants expected. For attractiveness, there was only a significant interaction effect between group and ratings, $F(1, 54) = 7.10$, $p = 0.01$, *partial* $\eta^2 = 0.12$, suggesting that HSA participants expected to be judged as less attractive than LSA participants expected. No main effects were found for attractiveness, all *p*s > 0.05. For nervousness, there was only a significant main effect of rating, $F(1, 54) = 102.96$, $p < 0.001$, *partial* $\eta^2 = 0.66$, indicating that all participants judged the peer as less nervous than they expected to be judged themselves. There was no effect of group and no interaction effect, all *p*s > 0.05. For the question 'Would you like to meet this person', there was only a significant interaction between group and ratings, $F(1, 54) = 6.15$, $p = 0.02$, *partial* $\eta^2 = 0.10$, showing that HSA participants would like to meet the peer more than that they expected that a peer would like to meet them, whereas LSA participants would like to meet the peer less than that they expected that a peer would like to meet them. There were no main effects, all *p*s > 0.05.



Supplementary figure 1. VAS scores of ratings of other (after viewing video) and self (before giving speech) on four questions for HSA and LSA participants.

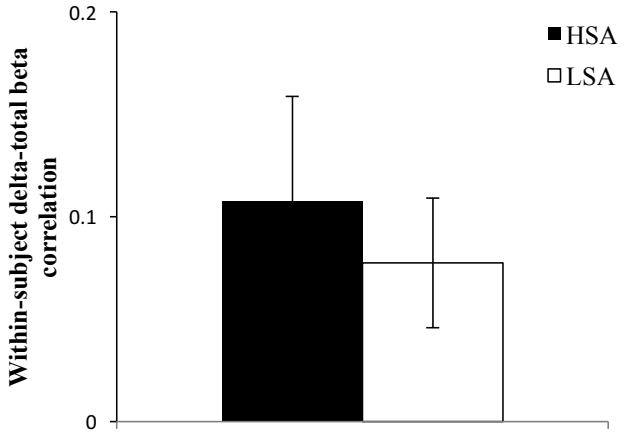
Note: ** $p < 0.01$ *** $p < 0.001$, error bars represent standard error.

Supplementary data 2

Within-subjects delta-beta correlation

We also extracted relative delta (1-4 Hz), total beta (14-30 Hz), low beta (14-20 Hz) and high beta (20-30 Hz) power per epoch, and calculated per participant the correlation between log-transformed delta power and log transformed total, low, or high beta power.

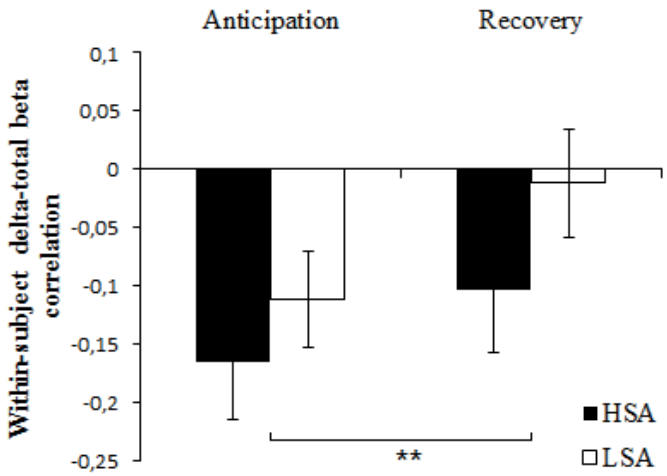
During resting, there was no difference in within-subject delta-beta correlation between HSA and LSA participants, F (1, 54) = 0.28, p = 0.60, η^2 = 0.01, F (1, 54) = 1.23, p = 0.27, η^2 = 0.02, F (1, 54) = 0.07, p = 0.80, η^2 = 0.001 (respectively total, low, and high beta, see Supplementary figure 2).



Supplementary figure 2. Within-subject correlation between relative delta and total beta power for HSA and LSA participants during resting state (error bars represent standard error of the mean).

During the social performance task, there was also no difference between HSA and LSA participants, F (1, 54) = 1.46, p = 0.23, *partial* η^2 = 0.03, F (1, 54) = 2.72, p = 0.11, *partial* η^2 = 0.05, F (1, 54) = 0.81, p = 0.37, *partial* η^2 = 0.02 (respectively total, low, and high beta). The interaction between Time (anticipation, recovery) and Group (HSA, LSA) was not significant, F (1, 54) = 0.36, p = 0.55, *partial* η^2 = 0.01, F (1, 54) = 0.13, p = 0.72, *partial* η^2 = 0.002, F (1, 54) = 1.61, p = 0.21, *partial* η^2 = 0.03 (respectively total, low, and high beta). Within-subject delta-beta correlation was more negative during anticipation than recovery for total beta, F (1, 54) = 7.19, p = 0.01, *partial* η^2 = 0.12, but not for low and high

beta, respectively F (1, 54) = 0.58, p = 0.45, *partial* η^2 = 0.01, F (1, 54) = 3.66, p = 0.06, *partial* η^2 = 0.06 (see Supplementary figure 3).



Supplementary figure 3. Within-subject correlation between relative delta and total beta power for HSA and LSA participants during the social performance task.

Note: ** p < 0.01, error bars represent standard error.

Chapter 4

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

This chapter is accepted for publication as:

Harrewijn, A., Van der Molen, M.J.W., Van Vliet, I.M., Houwing-Duistermaat, J.J., & Westenberg, P.M. (2017). Delta-beta correlation as a candidate endophenotype of social anxiety: A two-generation family study. *Journal of Affective Disorders, 227*, 398-405. doi: 10.1016/j.jad.2017.11.019

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.

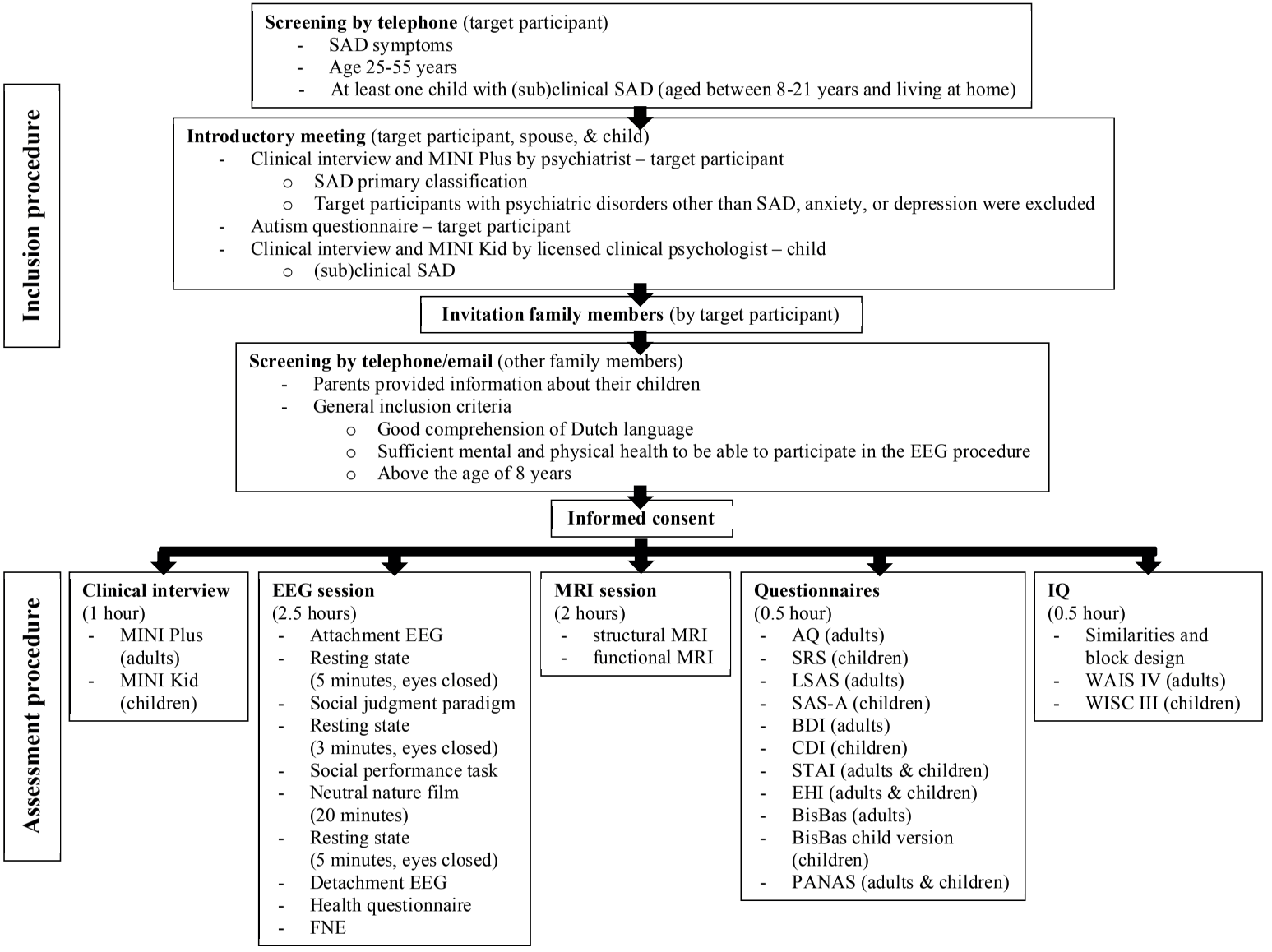

Inclusion procedure
Screening by telephone (target participant)
- SAD symptoms
- Age 25-55 years
- At least one child with (sub)clinical SAD (aged between 8-21 years and living at home)
Introductory meeting (target participant, spouse, & child)
- Clinical interview and MINI Plus by psychiatrist – target 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 $\beta = 0.51$, $p < 0.001$.

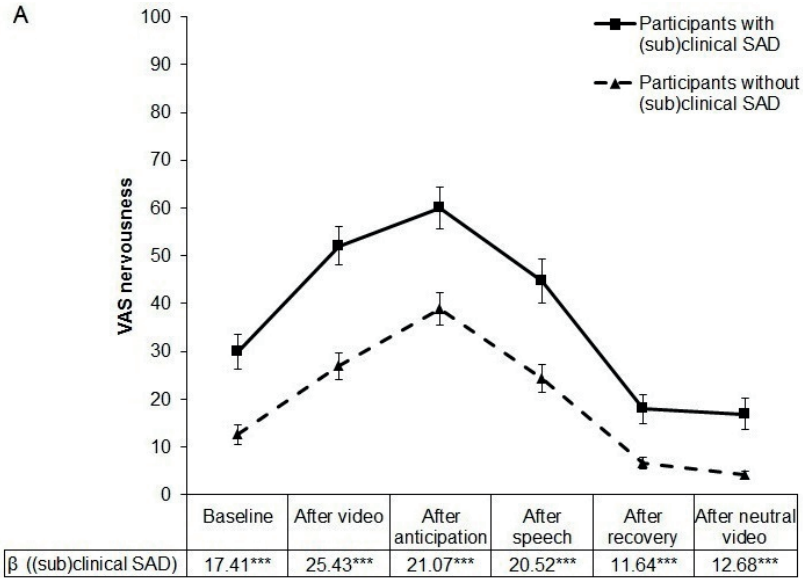
Table 1

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

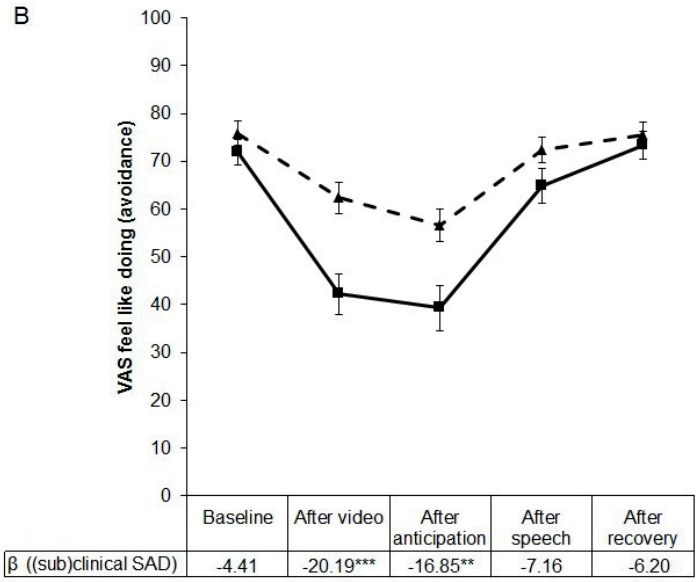
	Participants with SAD (12 females, 6 males)	Participants with subclinical SAD (10 females, 15 males)	Participants without (sub)clinical SAD (35 females, 35 males)
Age	39.67 (13.72)	21.36 (11.54)	29.99 (15.83)
Estimated IQ	106.67 (11.97)	103.00 (11.92)	105.96 (10.61)
Social anxiety (z-score)	3.83 (2.07)	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.

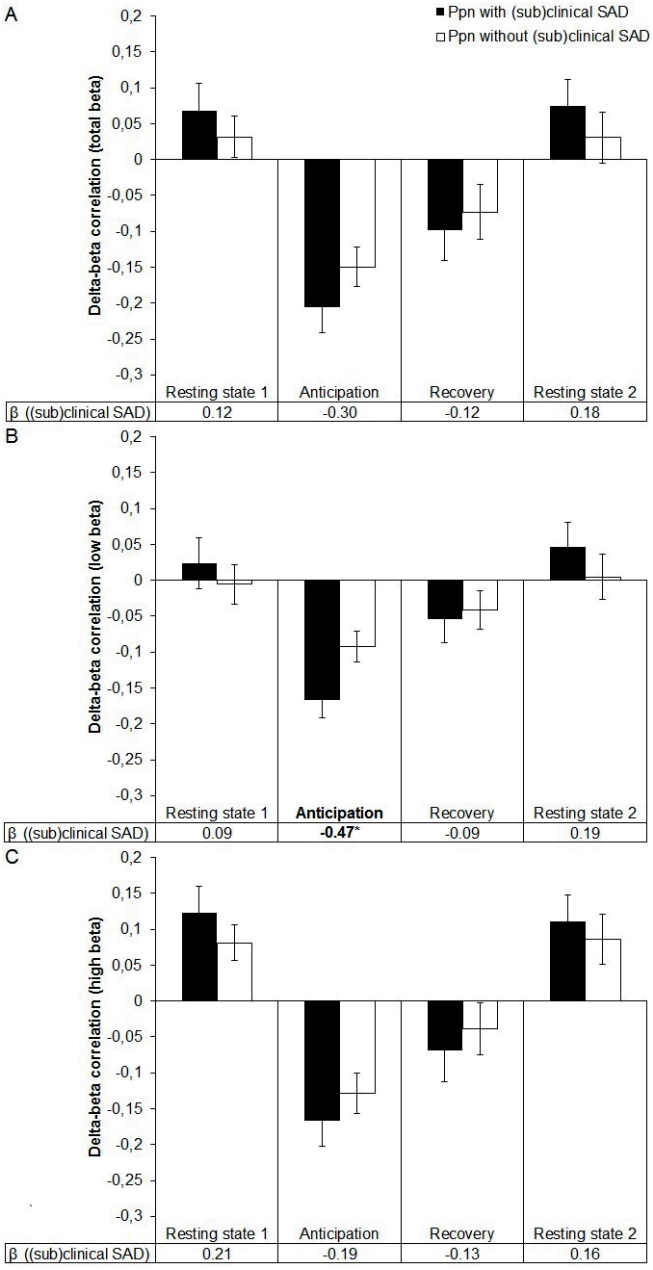


Figure 4. Correlation between delta and total (A), low (B), and high (C) beta power in participants with and without (sub)clinical SAD during the social performance task.

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]

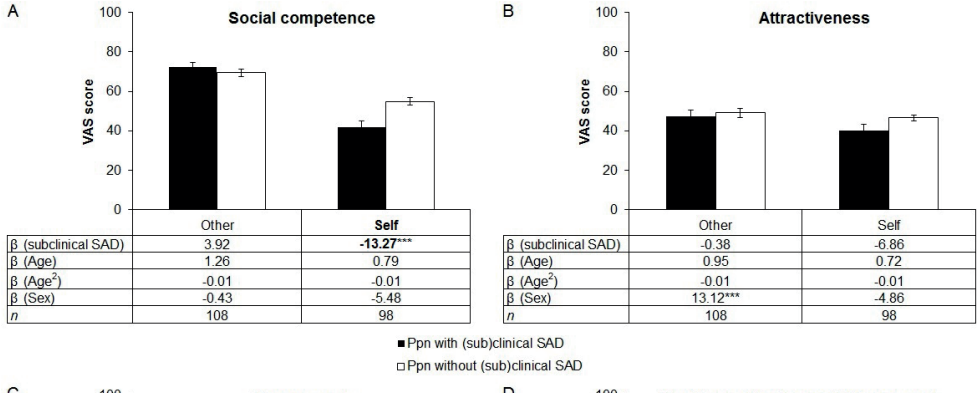
Note: RS1 = resting state 1; ANT = anticipation; REC = recovery; RS2 = resting state 2; T1 = time point 1.

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



	Other	**Self**
β (subclinical SAD)	3.92	**-13.27*****
β (Age)	1.26	0.79
β (Age^2)	-0.01	-0.01
β (Sex)	-0.43	-5.48
n	108	98

	Other	Self
β (subclinical SAD)	-0.38	-6.86
β (Age)	0.95	0.72
β (Age^2)	-0.01	-0.01
β (Sex)	13.12***	-4.86
n	108	98

	Other	**Self**
β (subclinical SAD)	-2.00	**13.44****
β (Age)	-2.43**	0.95
β (Age^2)	0.03	-0.02
β (Sex)	1.18	9.76
n	108	98

	Other	Self
β (subclinical SAD)	-0.52	-8.28
β (Age)	0.93	0.44
β (Age^2)	-0.01	-0.01
β (Sex)	10.15	-1.63
n	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])

Chapter 5

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

This chapter is submitted for publication as:

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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.

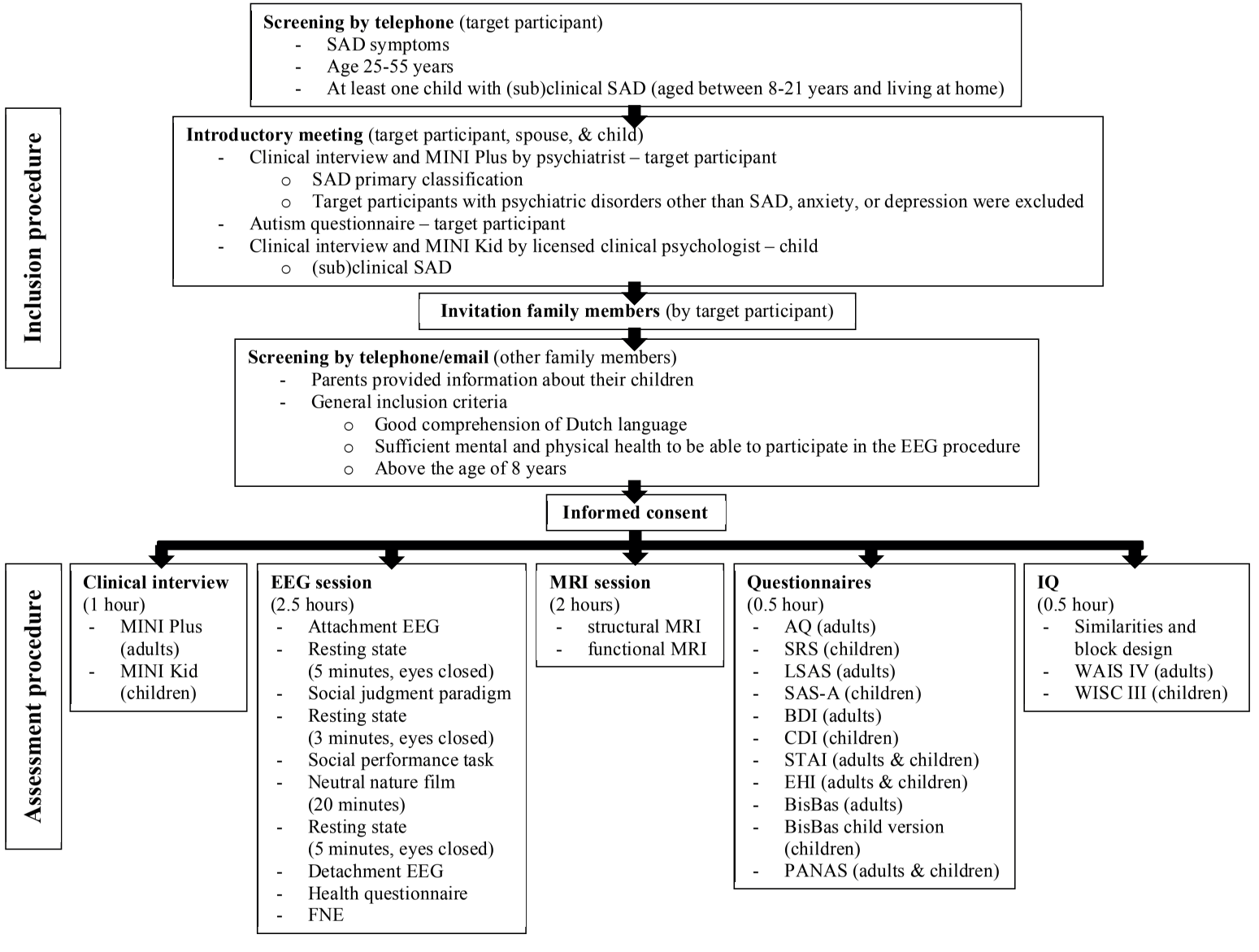

Inclusion procedure
Screening by telephone (target participant)
- SAD symptoms
- Age 25-55 years
- At least one child with (sub)clinical SAD (aged between 8-21 years and living at home)
Introductory meeting (target participant, spouse, & child)
- Clinical interview and MINI Plus by psychiatrist – target 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.

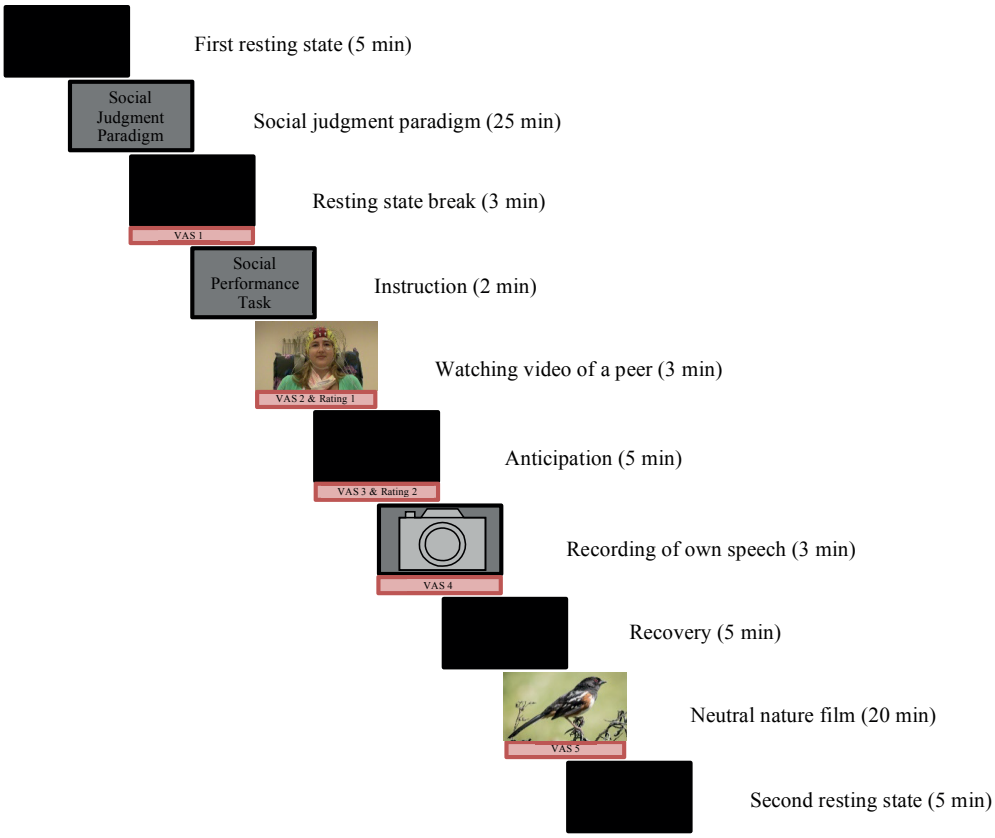


Figure 2. Overview of the social performance task.

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.

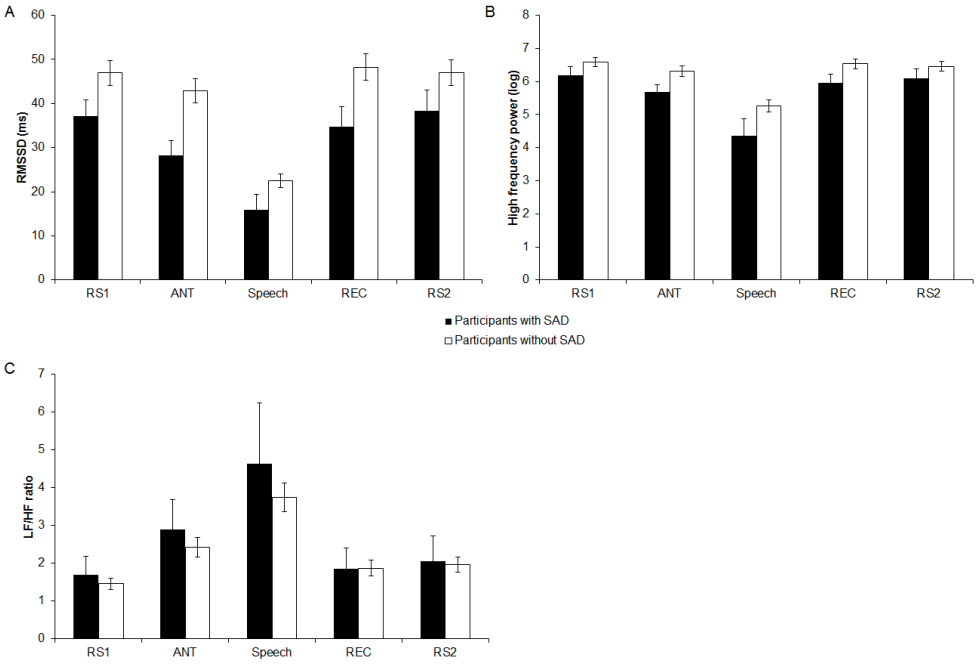


Figure 3. Uncorrected mean RMSSD (A), high frequency power (B), and LF/HF ratio (C) for participants with and without SAD during all five phases of the SPT.

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

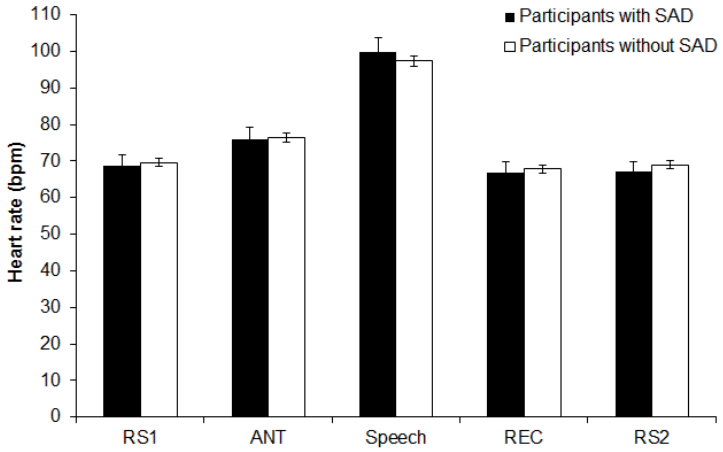
Overview of the percentage of deleted artefacts in HRV data for participants with and without SAD.

	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.

Chapter 6

Behavioral and EEG responses to social evaluation: A two-generation family study on social anxiety

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Abstract

Social anxiety disorder is an invalidating psychiatric disorder characterized by extreme fear and avoidance of one or more social situations in which patients might experience scrutiny by others. The goal of this two-generation family study was to delineate behavioral and electrocortical endophenotypes of social anxiety disorder related to social evaluation. Nine families of patients with social anxiety disorder (their spouse and children, and siblings of these patients with spouse and children) performed a social judgment paradigm in which they believed to be evaluated by peers. For each peer, participants indicated their expectation about the evaluative outcome, after which they received social acceptance or rejection feedback. Task behavior, as well as the feedback-related EEG brain potentials (N1, FRN, P3) and theta power were tested as candidate endophenotypes based on two criteria: co-segregation with social anxiety disorder within families and heritability. Results indicated that reaction time for indicating acceptance-expectations might be a candidate behavioral endophenotype of social anxiety disorder, possibly reflecting increased uncertainty or self-focused attention and vigilance during the social judgment paradigm. N1 in response to expected rejection feedback and P3 in response to acceptance feedback might be candidate electrocortical endophenotypes of social anxiety disorder, although the heritability analyses did not remain significant after correcting for multiple tests. Increased N1 possibly reflects hypervigilance to socially threatening stimuli, and increased P3 might reflect that positive feedback is more important for, and/or less expected by, participants with social anxiety disorder. Finally, increased feedback-related negativity and theta power in response to unexpected rejection feedback compared to the other conditions co-segregated with social anxiety disorder, but these EEG measures were not heritable. The candidate endophenotypes might play a new and promising role in future research on genetic mechanisms, early detection and/or prevention of social anxiety disorder.

Introduction

Social anxiety disorder (SAD) is a psychiatric disorder characterized by extreme anxiety and avoidance in one or more social situations (APA, 2013). SAD is a common and debilitating internalizing disorder (Furmark, 2002; Rapee & Spence, 2004), and a known precursor to other psychiatric disorders, such as depression and substance abuse disorders (Grant et al., 2005; Rapee & Spence, 2004; Spence & Rapee, 2016). The risk for developing SAD is higher for individuals with a close family member with SAD than for individuals without family members with SAD (Isomura et al., 2015), and 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). The genetic basis of psychiatric disorders could be studied by delineating endophenotypes, which are heritable trait markers in between the genotype and phenotype (Glahn et al., 2007; Gottesman & Gould, 2003; Iacono et al., 2016; Miller & Rockstroh, 2013). Electrocortical endophenotypes are specifically useful because they are presumably more closely related to genes than behavioral endophenotypes (Cannon & Keller, 2006). This study aims to delineate candidate endophenotypes of SAD by examining both behavioral and electrocortical responses to social evaluation.

The social judgment paradigm (SJP) (Gunther Moor, Van Leijenhorst, Rombouts, Crone, & Van der Molen, 2010; Somerville, Heatherton, & Kelley, 2006; Van der Molen et al., 2014) could be useful in delineating candidate endophenotypes of SAD because this task allows for examining behavioral and electrocortical responses to social evaluation. In this task, participants receive feedback that communicates social acceptance or rejection, which can either be congruent or incongruent with participants' expectancies (Van der Molen et al., 2014). At the behavioral level, a number of studies have shown an optimism bias in healthy participants, as they more often expect acceptance versus rejection feedback (Dekkers, Van der Molen, Gunther Moor, Van der Veen, & Van der Molen, 2015; Gunther Moor, Crone, & Van der Molen, 2010; Van der Molen et al., 2017; Van der Molen et al., 2014; Van der Veen, Van der Molen, Van der Molen, & Franken, 2016). Patients with SAD expected to be accepted less frequently than healthy controls before the 'Island Getaway task', a task in which participants received social feedback without indicating their expectation in each trial (Cao et al., 2015). This is in line with cognitive-behavioral studies showing that patients with SAD expect negative outcomes of social situations (Clark & McManus, 2002; Hirsch & Clark, 2004). In SAD, the SJP has not been studied yet. Fear of negative evaluation has been studied using the SJP in healthy females, and was not related to feedback expectations during

the task (Van der Molen et al., 2014). Notably, fear of negative evaluation was positively correlated with reaction time for indicating feedback expectations in healthy females, suggesting increased self-focused attention and vigilance during the SJP (Van der Molen et al., 2014). So, both feedback expectations and reaction time to indicate these expectations might be candidate endophenotypes of SAD.

At the electrocortical level, two event-related potentials (ERPs) have been examined using the SJP: the feedback-related negativity (FRN) and P3. The FRN (a negative component around 250 ms after feedback) is typically increased for feedback that is unexpected or reflecting poor performance (Ferdinand et al., 2012; Oliveira et al., 2007; Van Noordt & Segalowitz, 2012). However, it is unknown whether the FRN in response to social feedback is modulated by social anxiety in the SJP. There was no relation between fear of negative evaluation and FRN in healthy females (Van der Molen et al., 2014). In the Island Getaway task, the FRN was increased after acceptance feedback in patients with SAD (Cao et al., 2015), whereas FRN was increased after rejection feedback in healthy children with higher levels of parent-reported social anxiety (Kujawa et al., 2014). The effect of social anxiety on feedback valence might be related to feedback expectancies during the task, but this was not assessed on a trial-by-trial basis in the Island Getaway task (Cao et al., 2015; Kujawa et al., 2014). Thus, using the SJP allows for delineating the (differential) effect of feedback valence (acceptance versus rejection) and congruency (expected versus unexpected) on electrocortical responses that might be related to SAD. If there is indeed an effect of valence of social evaluative feedback in social anxiety (Cao et al., 2015; Kujawa et al., 2014), this should be present on both expected and unexpected trials of the SJP.

The P3 (a positive component that peaks around 300-500 ms after stimulus onset) is known to be sensitive to emotionally motivational stimuli (Hajcak et al., 2013). P3 results for healthy participants in the SJP are mixed. Some have found that the P3 was largest in response to expected acceptance feedback, and suggested that this P3 response might be related to the level of reward communicated by expected acceptance feedback (Van der Veen et al., 2016; Van der Veen, Van der Molen, Sahibdin, & Franken, 2014). However, other studies did not find this P3 effect (Dekkers et al., 2015; Van der Molen et al., 2014). Further, P3 amplitude was not associated with fear of negative evaluation in healthy participants in the SJP (Van der Molen et al., 2014), nor with SAD in the Island Getaway task (Cao et al., 2015). If the social feedback-related P3 indeed reflects reward processing (Van der Veen et al., 2016; Van der Veen et al., 2014), the P3 in response to expected acceptance feedback might be a candidate endophenotype of SAD, based on altered reward-system reactivity in social anxiety

(Cremers, Veer, Spinhoven, Rombouts, & Roelofs, 2015; Lahat, Benson, Pine, Fox, & Ernst, 2016). But, if the social feedback-related P3 rather reflects the processing of emotionally motivational stimuli (Hajcak et al., 2013), the P3 in response to expected and unexpected acceptance feedback might be a candidate endophenotype of SAD, given the importance of positive social evaluation for patients with SAD (Rapee & Heimberg, 1997).

More recently, studies using the SJP have examined neural oscillatory power in response to social evaluation (Van der Molen et al., 2017; Van der Veen et al., 2016). In contrast to ERPs, time-frequency power represents neural activity that is not phase-locked to the onset of a stimulus and this can yield additional insights into the neural dynamics (M. X. Cohen, 2014; Makeig et al., 2004; Van der Molen et al., 2017; Van Noordt, Campopiano, & Segalowitz, 2016). Theta oscillatory power seems sensitive to social threat (Cristofori et al., 2013; Van Noordt, White, Wu, Mayes, & Crowley, 2015), and recent SJP studies have reported higher theta power in response to unexpected rejection feedback in healthy participants (Van der Molen et al., 2017; Van der Veen et al., 2016). Although theta power has not yet been studied in social anxiety, increased theta power in response to unexpected rejection feedback might be a candidate endophenotype of SAD, reflecting increased sensitivity to negative feedback in SAD (Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004).

It is argued that endophenotypes could play an important role in understanding the genetic mechanisms underlying SAD (Cannon & Keller, 2006; Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013), because their genetic basis is proposed to be simpler than the genetic basis of complex psychiatric disorders (Cannon & Keller, 2006; Glahn et al., 2007). To meet the criteria of an endophenotype of SAD, behavioral and electrocortical responses to social evaluation should adhere to certain criteria: (1) association with SAD, (2) co-segregation with SAD within families, (3) heritability, (4) state-independence, and (5) increased in non-affected family members compared to the general population (Glahn et al., 2007; Gottesman & Gould, 2003). The first criterion could be studied by comparing patients with SAD and healthy controls (as in Cao et al. (2015)). The second and third criterion are based on the observation that psychiatric disorders run in families (Glahn et al., 2007; Gottesman & Gould, 2003). Within these families, the endophenotype should be displayed by persons with the disorder ('co-segregation'). Furthermore, the endophenotype should be heritable. The fourth criterion indicates that persons with the disorder should display the endophenotype whether or not the illness is active (Gottesman & Gould, 2003). The fifth

criterion could be studied by comparing family members of patients with SAD with healthy controls.

Although various methods have been used to examine the endophenotype criteria, a family design seems particularly appropriate to assess both the 'co-segregation' and 'heritability' criteria of an endophenotype. Extended families (e.g. including partner and children of patient, and siblings of patient with their partner and children) provide the opportunity to compare family members with and witout SAD ('co-segregation'). Furthermore, we examined extended families instead of twins or sib-pairs, to increase the power to identify genetic variability within the family (because of the many different genetic relations) and thus heritability (Gur et al., 2007; Williams & Blangero, 1999). Moreover, we selected families based on two probands (adult with SAD and child with (sub)clinical SAD) to ensure we focused on a genetic 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).

The goal of the current study was to investigate for the first time whether behavioral and electrocortical responses to social evaluation are candidate endophenotypes of SAD. In our two-generation family study, patients with SAD and their family members performed the SJP to assess behavioral and electrocortical responses to social evaluation. For the behavioral data, we expected that the number of trials on which participants expected social acceptance, as well as the corresponding reaction time for indicating feedback expectations are candidate endophenotypes, because previous studies have confirmed the first criterion for endophenotypes ('association') (Cao et al., 2015; Van der Molen et al., 2014). Even though the SJP has not been studied in SAD before, we expected the following electrocortical endophenotypes of SAD: the FRN in response to valence regardless of expectations (Cao et al., 2015; Kujawa et al., 2014), altered P3 in response to expected acceptance feedback (Cremers, Veer, Spinhoven, Rombouts, & Roelofs, 2015; Lahat et al., 2016; Van der Veen et al., 2016; Van der Veen et al., 2014) or to expected and unexpected acceptance feedback (Hajcak et al., 2013; Rapee & Heimberg, 1997), and increased theta power in response to unexpected rejection feedback (Van der Molen et al., 2017; Van der Veen et al., 2016). We exploratively tested whether the N1 might be a candidate endophenotype of SAD, even though it has not been studied before in the SJP, because it was found during visual inspection of the data and might be related to early attentional processes such as hypervigilance to socially threatening stimuli (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013).

Methods

Participants

This was the first study intensively investigating patients with SAD and their family members. Families were recruited via media exposure (radio, tv, newspapers) and selected based on two probands: one adult with SAD ('target participant') and his/her child with clinical or subclinical SAD (further referred to as '(sub)clinical'). SAD was diagnosed by a psychiatrist using a clinical interview and the structured Mini-Plus International Neuropsychiatric Interview (Sheehan et al., 1998; Van Vliet & De Beurs, 2007), based on the DSM-IV-R criteria for SAD generalized subtype. The psychiatrist confirmed that these patients also met the 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)). In the target's child, (sub)clinical SAD was diagnosed by a licensed clinician using a clinical interview and the structured MINI Kid interview (Bauhuis et al., 2013; Sheehan et al., 2010).

Nine target participants were included with their spouse and children, and the siblings of the target participant with spouse and children (total n = 132). Figure 1 depicts the inclusion criteria. Nine participants only filled out questionnaires at home, five participants only participated in EEG resting state, and data of one participant could not be collected due to technical problems. One participant was excluded because s/he did not believe the cover story and one because s/he fell asleep during the task. Analysis of the SJP was based on 115 participants (59 females, M_{age} = 30.29, SD = 15.57, range = 8-61 years).

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 computed by simulating 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 et al., 2008). We included somewhat fewer families, since the families we included were larger (on average 14.67 instead of 10 members per family) which results in more power than smaller families (Dolan et al., 1999; Gur et al., 2007; Rijsdijk et al., 2001; Williams & Blangero, 1999).

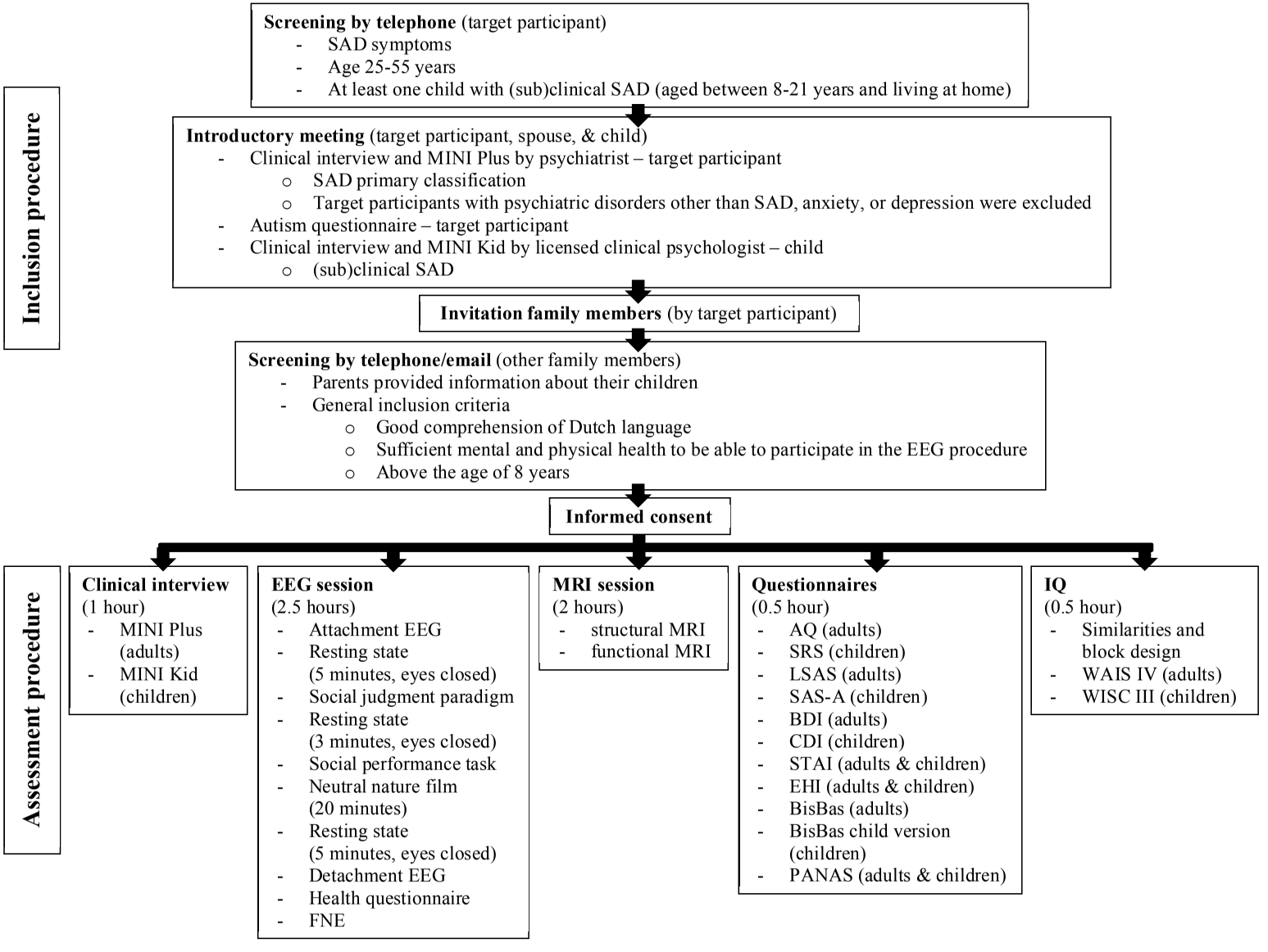

Inclusion procedure
Screening by telephone (target participant)
- SAD symptoms
- Age 25-55 years
- At least one child with (sub)clinical SAD (aged between 8-21 years and living at home)
Introductory meeting (target participant, spouse, & child)
- Clinical interview and MINI Plus by psychiatrist – target 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 procedure of the Leiden Family Lab study on SAD. All family members participated in all parts of the assessment procedure in one or two days. The order of these parts differed between participants, depending on their preferences and availability of the labs. Mostly, participants came to the lab with their family. 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). Results of the social performance task are reported in Harrewijn, Van der Molen, Van Vliet, Houwing-Duistermaat, et al. (in press). Participants did not know beforehand about the social performance task.

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).

Experimental design

Figure 1 shows a flow-chart of the inclusion and assessment procedure of the Leiden Family Lab study on SAD (Harrewijn, Van der Molen, Van Vliet, Houwing-Duistermaat, et al., in press). All participants received €75 for their participation and we reimbursed travel expenses. All participants provided written informed consent, according to the Declaration of Helsinki (1991). Both parents signed the informed consent form for their children from 8-18 years of age, children of 12-18 years also signed themselves. The procedure was approved by the medical ethics committee of the Leiden University Medical Center.

Social judgment paradigm

We used the SJP as described in Van der Molen et al. (2014, 2017) (Figure 2). When participants were contacted to make an appointment for the EEG session, we asked them to email us a portrait photograph of themselves for a task about first impressions. We told them a panel of peers would evaluate their photograph and indicate whether they liked or disliked the person on the picture. This was actually a cover story to elicit feelings of social evaluation during the task. Most participants sent their photograph at least one week before the EEG session (31 participants sent their photograph 2-6 days before the EEG session). Participants were reminded of this cover story right before the SJP.

The SJP consisted of 10 practice trials and 150 experimental trials in three blocks with a short break in between. Each trial started with a fixation cross (jittered duration of 500-1000 ms). Then, the picture of a peer[8] appeared and remained on the screen during the rest of the trial. Participants had to indicate whether this person would like or dislike them by pressing the button in the left or right arm rest (the meaning of the buttons was counterbalanced between participants). The response of the participants (yes or no) was immediately shown on the left side of the picture. If the participants did not respond within 3000 ms, the message 'too slow' appeared and these trials were excluded from analysis. After a delay (3000 ms), the feedback of the peer was shown on the right side of the picture for 2000 ms. Before and after the SJP, participants were asked to indicate on a visual analogue scale (VAS) from 0 (exclusively negative) to 100 (exclusively positive) how they expected to be evaluated (before), and how they were evaluated (after) (similar to Cao et al. (2015)). Afterwards, we asked participants not to tell their family members about the SJP. All but one participant reported that they believed the cover story of the SJP (this participant was excluded).

[8] We used a 17-inch computer monitor (60Hz refresh rate, visual angle (width/height) = 4.66 x 6.05) and Eprime 2.0 stimulus presentation software (Psychology Software Tools, Pittsburgh, PA, USA). We used 5 different sets of pictures, for 5 different age categories (8-12, 13-17, 18-25, 26-39, 40-55 years), to make sure that all participants were evaluated by peers. All faces (50% female) were showing a neutral expression, as validated with the Self-Assessment Manikin (Bradley & Lang, 1994) in the 5 age categories (n = 20 per age category).

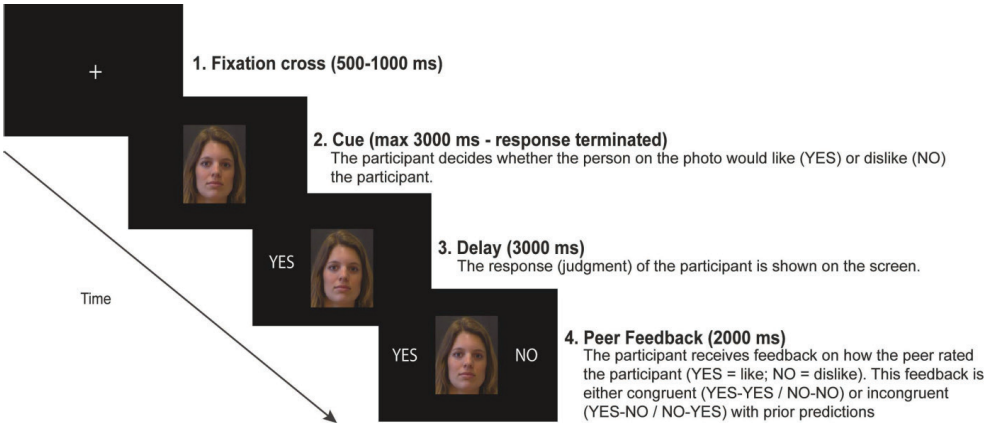


Figure 2. Trial sequence of the social judgment paradigm.

Reprinted from NeuroImage, 146, Van der Molen, M.J.W., Dekkers, L.M.S., Westenberg, P.M., Van der Veen, F.M., & Van der Molen, M.W., Why don't you like me? Midfrontal theta power in response to unexpected peer rejection feedback, 474-783, Copyright (2017), with permission from Elsevier.

EEG recording and signal processing

EEG was recorded at a sampling rate of 1024 Hz with the BioSemi Active Two system (Biosemi, Amsterdam, The Netherlands) from 64 Ag-AgCl electrodes mounted in an elastic electrode cap (10/20 placement). The ground electrode was replaced by a feedback loop consisting of the common mode sense and driven right leg electrode. The common mode sense was used as online reference. Eight external electrodes were used: two for horizontal electrooculography (placed at left and right canthus), two for vertical electrooculography (placed 1 cm above and below the left eye), two for offline re-referencing (placed at mastoids), and two for measuring heart rate (modified lead-2 placement on chest; results will be reported elsewhere).

During offline preprocessing in BrainVision Analyzer (Brain Products GmbH), the signal was down sampled to 512 Hz, re-referenced to the average of left and right mastoids, and band-pass filtered (0.5-40 Hz, 50 Hz notch). Valid response segments (4000 ms before and after feedback) were selected if there was no response in the first 100 ms after the picture appeared, and if there was no second response 500 ms after the first response (which might indicate uncertainty of the answer of the participant). These segments were manually inspected for artifacts and noisy channels were interpolated. Then, ocular artifacts were adjusted for by ocular independent component analysis. The segments were also

automatically checked for artifacts[9]. If an artifact was detected in one channel, the entire segment was removed during both manual and automatic artifact detection. Supplementary table 1 shows the number of artifact-free trials per condition for participants with and without SAD, these trials were used for both ERP and time-frequency analyses. Participants with SAD had more artifact-free trials overall, $\beta = 7.25$, $p = 0.02$, and more artifact-free trials indicating they expected to be disliked, whereas participants without SAD had more clean trials indicating they expected to be liked, $\beta = -13.88$, $p < 0.001$.

ERP analysis. We created ERP segments of 1200 ms (200 ms before feedback and 1000 ms after feedback), which were baseline corrected (-200 – 0 ms) and averaged across trials (for the four conditions separately). For each component, we manually selected three electrodes with the largest peak amplitude from the grand-grand average (including all participants and conditions, as recommended by Kappenman and Luck (2016)), and tested with a repeated-measures ANOVA which electrode showed the largest peak amplitude. We continued analysis with this single midline electrode.

The FRN was computed by subtracting P2 amplitude from the subsequent negative peak (peak-to-peak method) (Dekkers et al., 2015; Van der Molen et al., 2014). The automatic peak detection procedure (local maximum) was used to determine the P2 240-340 ms after feedback, and the subsequent negative peak 290-390 ms after feedback. The peaks were manually adjusted if the P2 did not precede the most negative peak. The FRN was maximal at AFz, Fz, and FCz. Since FRN amplitude did not differ between these channels, we used FRN amplitude from Fz for further analyses (Dekkers et al., 2015; Van der Veen et al., 2016). P3 amplitude was determined via an area measure (Luck, 2005) between 360 and 500 ms, based on the grand-grand average (Kappenman & Luck, 2016). P3 was largest on Pz, compared to Fz and Cz.

When visually inspecting the data, we encountered an early negative component that peaked around 180 ms after feedback and seemed to differ between participants with and without SAD. This was not in line with the unbiased method as proposed by Kappenman and Luck (2016), but we decided to exploratively study this component further. We termed this component the N1 and we used an area measure between 130-220 ms. N1 amplitude was most negative at FCz, compared to AFz and Fz.

[9] We used the following criteria during automatic artifact rejection: maximal allowed voltage step: 50 μV/ms; maximal allowed absolute difference: 200 μV with interval length of 200 ms; lowest allowed activity in intervals: 0.5 μV with interval length of 100 ms.

Time-frequency analysis. We performed time-frequency analysis on the same artifact-free trials as the ERP analysis. We created segments of 8000 ms (4000ms before and after feedback). We applied a current-source density transformation, and a baseline correction using a 2100-2400 ms post-feedback baseline interval (due to the jittered duration of the fixation cross it was not possible to use a consistent pre-feedback baseline). Time-frequency characteristics were extracted from the EEG data by convolution of the single trials with complex Morlet wavelets (Gaussian-windowed sine waves), which increased from 1 to 40 Hz in 40 logarithmically spaced steps. We applied a Morlet parameter of 5, and the unit energy normalization method. Trials were averaged for the four conditions separately. Data was normalized according to the ratio change compared to the 2100-2400 ms post-feedback baseline interval. Theta power (layer 16-23, 4.13-8.01 Hz) was extracted from a 300-500 ms window after feedback onset from the AFz, Fz and FCz electrodes (Van der Molen et al., 2017). We used data from Fz for further analyses as this electrode yielded largest theta values (as indicated by the grand-grand average (Kappenman & Luck, 2016)).

Statistical analysis

First, we validated the differences between participants with and without SAD by comparing their self-reported symptoms of social anxiety (La Greca & Lopez, 1998; Liebowitz, 1987), fear of negative evaluation (Carleton et al., 2006), and depression (Beck et al., 1996; Kovacs, 1992). For social anxiety and depressive symptoms, we computed z-scores based on normative samples (Fresco et al., 2001; Inderbitzen-Nolan & Walters, 2000; Miers et al., 2014; Roelofs et al., 2013) to enable comparison 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 (standardized), age (standardized)^2 and sex as independent variables. A random effect was included to take into account the genetic correlations between family members.

Second, we tested whether behavioral and electrocortical measures during the SJP were candidate endophenotypes of SAD, using the two criteria 'co-segregation with SAD within families' and 'heritability' (Glahn et al., 2007). Like previous studies, we calculated a bias score indicating the percentage of trials on which participants expected to be accepted by peers (number of acceptance-expectations/(number of acceptance + rejection expectations)*100). Co-segregation analyses were performed by fitting regression models with the candidate endophenotype as dependent variable, and SAD, congruency, feedback, congruency*feedback, SAD*congruency, SAD*feedback, SAD*congruency*feedback, age

(standardized), age (standardized)^2 and sex as independent variables[10]. Random effects were included for the genetic correlations between family members and the correlations between conditions within a person. A significance level of $\alpha = 0.05$ was used for statistical analyses.

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 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) (Almasy & Blangero, 2010). Age (standardized), age (standardized)^2 and sex were included as covariates, and were removed from the final model if $p > 0.05$. We could not run bivariate analyses to calculate genetic correlations between the candidate endophenotypes and SAD, because too few non-target participants were diagnosed with SAD. Since the assumptions for SOLAR (trait standard deviation higher than 0.5, residual kurtosis normally distributed) were not met for most variables, an inverse normal transformation was applied to all EEG variables in this step, as implemented in SOLAR (Almasy & Blangero, 1998, 2010). We applied a Bonferroni correction for performing multiple (25) heritability tests (i.e. $\alpha = 0.002$ as threshold for declaring statistical significance).

An important issue in analyzing the data from this family design is ascertainment. That is, we selected families based on a specific criterion (SAD) that is related to the candidate endophenotypes, which could influence the results. However, SAD was included as an independent variable in the co-segregation analyses, which is sufficient to correct for ascertainment (Monsees et al., 2009). In the heritability analyses, we corrected for ascertainment by using the proband correction available in the SOLAR software (Almasy & Blangero, 1998). Basically, SOLAR corrects for ascertainment by subtracting the likelihood of the probands from the likelihood of the rest of the sample (De Andrade & Amos, 2000; Hopper & Mathews, 1982). In this study, the target participant with SAD and his/her child with (sub)clinical SAD were indicated as 'probands' in SOLAR.

[10] The interaction term congruency*feedback focused on unexpected rejection versus the other conditions, because this condition is hypothesized as the most 'painful' condition (Gunther Moor et al., 2010a; Van der Molen et al., 2017). For N1, we recoded the variables in such a way that the congruency*feedback interaction focused on expected acceptance, based on visual inspection of the data.

Results

Participant characteristics

Participants with SAD were older than participants without SAD, but there was no difference in estimated IQ between participants with and without SAD (Table 1). None of the participants with SAD were diagnosed with a current depressive episode, further descriptives of the groups in terms of clinical diagnoses can be found in Supplementary table 2. We validated the differences between participants with and without SAD, by showing that participants with SAD reported more symptoms of social anxiety, fear of negative evaluation, and depression compared to participants without SAD.

Table 1

Uncorrected means (and standard errors) of participants with and without SAD on the self-report questionnaires.

	Participants with SAD (12 females, 6 males)	Participants without SAD (47 females, 50 males)	β	*p*
Age	39.67 (3.24)	28.55 (1.56)	0.69	0.01
Estimated IQ	106.67 (2.82)	105.26 (1.13)	-0.83	0.76
Social anxiety (z-score)	3.83 (0.49)	0.40 (0.14)	3.10	< 0.001
Fear of negative evaluation	31.89 (2.73)	13.49 (0.85)	18.69	< 0.001
Depression (z-score)	0.44 (0.20)	-0.47 (0.07)	0.98	< 0.001

Note: SAD = social anxiety disorder

Behavioral data

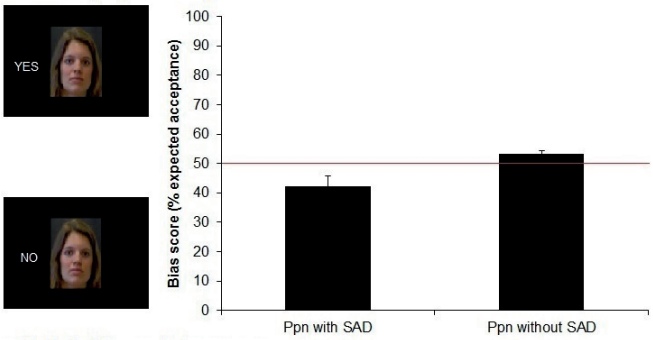
We tested whether the behavioral measures during the SJP were candidate endophenotypes of SAD (Figure 3 and Supplementary table 3). Co-segregation analysis with bias score revealed that participants with SAD expected rejection more often during the SJP than participants without SAD, $\beta = -14.39$, $p < 0.001$. Co-segregation analysis with VAS ratings revealed that participants with SAD expected rejection more often before the SJP and had experienced rejection more often after the SJP than participants without SAD, $\beta = -12.05$, $p = 0.04$. All participants had experienced rejection more often after the SJP, than they had expected before the SJP, $\beta = -12.88$, $p < 0.001$.

In addition, participants with SAD were overall slower than participants without SAD with indicating their expectations during the SJP, $\beta = 260.66$, $p = 0.01$. However, this was less the case for rejection-expectations, as indicated by the significant interaction between SAD

and condition, $\beta = -135.78$, $p < 0.001$. The main effect of condition, $\beta = 31.25$, $p = 0.052$, was probably driven by this interaction. Heritability analyses showed that none of the behavioral measures were heritable, the heritability estimate of reaction time for acceptance-expectations was only significant if we did not correct for multiple tests, $h^2 = 0.28$, $p = 0.02$.

A Dependent variable: bias score

1. Co-segregation analysis

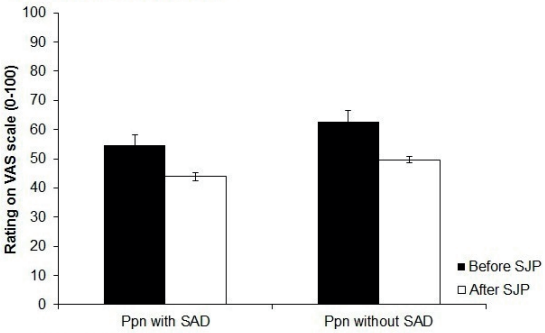


2. Heritability analysis

	h^2	SE	p	p (Age)	p (Age^2)	p (Sex)
Bias score	0.17	0.12	0.06	0.002	0.17	0.85

B Dependent variable: VAS ratings

1. Co-segregation analysis

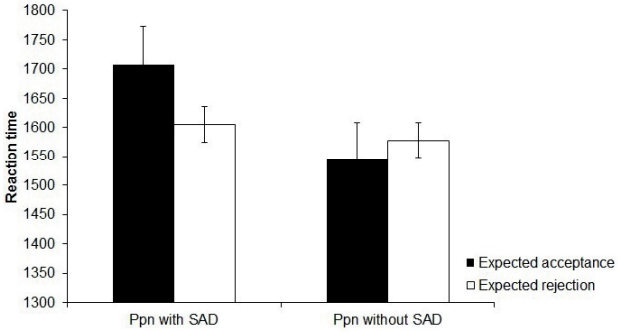


2. Heritability analysis

	h^2	SE	p	p (Age)	p (Age^2)	p (Sex)
Rating before SJP	0.12	0.16	0.17	0.03	0.002	0.53
Rating after SJP	0.00	-	0.50	0.01	0.83	0.46

C Dependent variable: reaction time

1. Co-segregation analysis



2. Heritability analysis

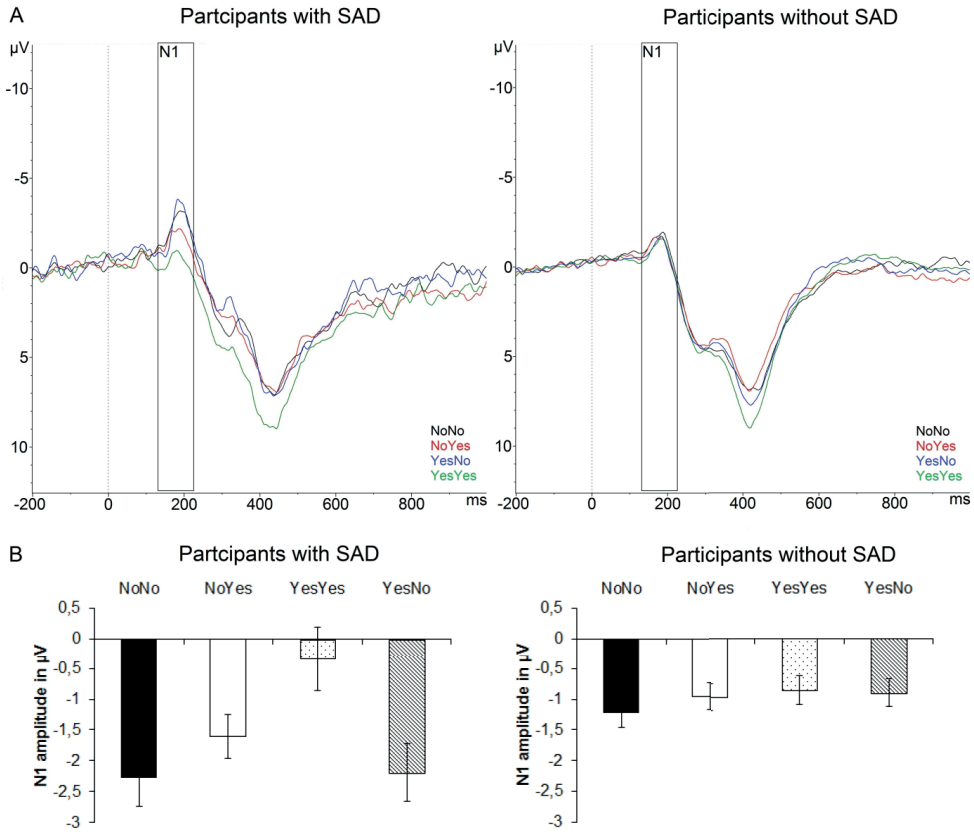
	h^2	SE	p	p (Age)	p (Age^2)	p (Sex)
RT acceptance	0.28	0.16	0.02	0.02	0.03	0.11
RT rejection	0.25	0.22	0.11	< 0.001	0.01	0.16

Figure 3. Means (1) and heritability analysis (2) for bias score (A), VAS ratings before and after the SJP (B), and reaction time for indicating acceptance and rejection-expectations for participants with and without SAD. Heritability results did not remain significant after correction for performing multiple tests.

Note: SAD = social anxiety disorder; h^2 = heritability; SE = standard error; VAS = visual analogue scale; SJP = social judgment paradigm; RT = reaction time.

ERP data

N1. We exploratively studied the N1 component as a candidate endophenotype of SAD (Figure 4 and Supplementary table 3). N1 and SAD co-segregated within families, $\beta = -1.24$, $p = 0.01$, with participants with SAD showing increased N1 across all conditions. The interaction between SAD and valence, $\beta = 1.03$, $p = 0.02$, was probably driven by the three-way interaction between SAD, congruency and feedback, $\beta = 0.52$, $p < 0.001$, showing that N1 was increased in all conditions except after expected acceptance feedback in participants with SAD. Heritability analyses revealed that N1 was not heritable, the heritability estimate of N1 after expected rejection feedback was only significant if we did not correct for multiple tests, $h^2 = 0.40$, $p = 0.03$.



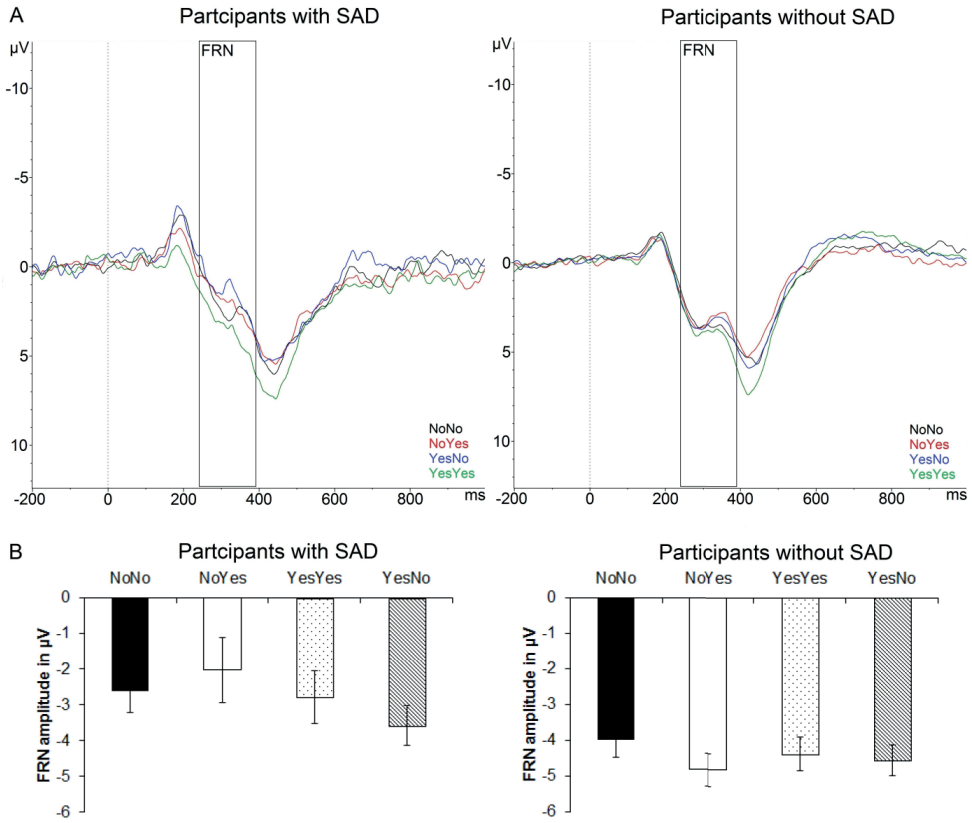
C Heritability analysis

	h^2	SE	p	p (Age)	p (Age^2)	p (Sex)
NoNo	0.40	0.29	0.03	0.15	0.03	0.09
NoYes	0.46	0.54	0.09	0.02	0.001	0.49
YesYes	0.00	-	0.50	0.06	0.02	0.21
YesNo	0.06	0.13	0.31	0.002	< 0.001	0.93
YesYes vs Rest	0.01	0.11	0.48	0.50	0.81	0.65

Figure 4. ERP waves for the four conditions (negative values plotted upwards) (A), N1 amplitude for participants with and without SAD (B), and results of the heritability analysis (C). N1 was computed as an area measure in the time window from 130 to 220 ms at electrode FCz. Heritability results did not remain significant after correction for performing multiple tests.

Note: SAD = social anxiety disorder; NoNo = expected rejection; NoYes = unexpected acceptance; YesYes = expected acceptance; YesNo = unexpected rejection; h^2 = heritability; SE = standard error. SE could not be computed if $h^2 = 0$.

FRN. There was no co-segregation within families between SAD and FRN across conditions (Figure 5 and Supplementary table 3). The two-way interaction between congruency and valence, $\beta = 0.60$, $p = 0.03$, revealed that FRN was increased after unexpected rejection feedback compared to the other conditions. This effect was increased in SAD, as indicated by the three-way interaction between SAD, congruency and feedback, $\beta = -0.94$, $p < 0.001$. Heritability analyses revealed that FRN was not heritable, the heritability estimates of FRN during expected and unexpected rejection feedback were only significant if we did not correct for multiple tests, respectively $h^2 = 0.48$, $p = 0.01$ and $h^2 = 0.36$, $p = 0.02$. FRN during unexpected rejection compared to the other conditions was not heritable, $h^2 = 0.002$, $p = 0.49$.



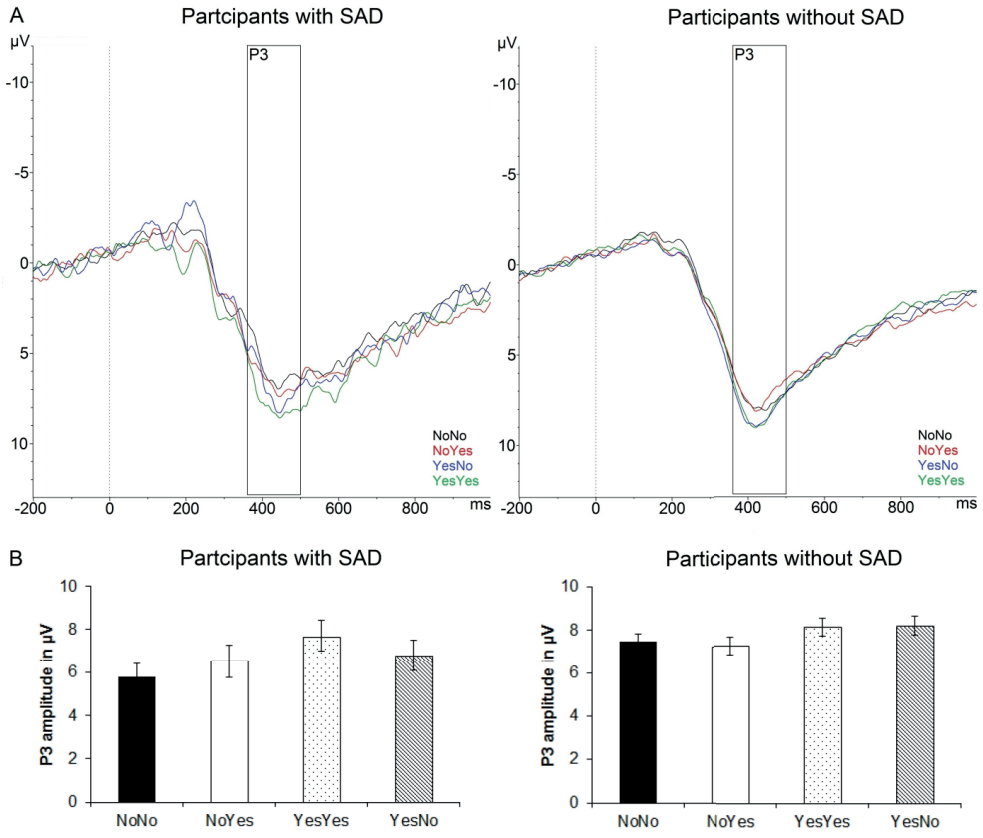
C Heritability analysis

	h^2	SE	p	p (Age)	p (Age^2)	p (Sex)
NoNo	0.48	0.25	0.01	< 0.001	0.002	0.14
NoYes	0.00	-	0.50	< 0.001	0.03	0.17
YesYes	0.10	0.16	0.23	< 0.001	< 0.001	0.02
YesNo	0.36	0.25	0.02	< 0.001	0.005	0.02
YesNo vs Rest	0.002	0.11	0.49	0.51	0.46	0.56

Figure 5. ERP waves for the four conditions (negative values plotted upwards) (A), FRN amplitude for participants with and without SAD (B), and results of the heritability analysis (C). FRN was computed using the peak-to-peak method in the time windows 240-340 and 290-390 ms at electrode Fz. Heritability results did not remain significant after correction for performing multiple tests.

Note: SAD = social anxiety disorder; NoNo = expected rejection; NoYes = unexpected acceptance; YesYes = expected acceptance; YesNo = unexpected rejection; h^2 = heritability; SE = standard error. SE could not be computed if $h^2 = 0$.

P3. There was no co-segregation within families between SAD and P3 across conditions (Figure 6 and Supplementary table 3). Overall, there was an effect of congruency, $\beta = -0.94$, $p = 0.001$, and of valence, $\beta = -0.68$, $p = 0.01$. The interaction between SAD and valence, $\beta = -1.02$, $p = 0.03$, showed that P3 was increased after acceptance compared to rejection feedback for participants with SAD, but not for participants without SAD. Heritability analyses revealed that P3 was not heritable, the heritability estimates of P3 in response to expected and unexpected acceptance feedback were only significant if we did not correct for multiple tests, respectively $h^2 = 0.38$, $p = 0.01$ and $h^2 = 0.41$, $p = 0.01$.



C Heritability analysis

	h^2	SE	p	p (Age)	p (Age^2)	p (Sex)
NoNo	0.15	0.19	0.21	0.63	0.01	0.95
NoYes	0.41	0.20	0.01	0.88	< 0.001	0.91
YesYes	0.38	0.18	0.01	0.82	0.004	0.70
YesNo	0.07	0.21	0.36	0.58	0.12	0.47
YesNo vs Rest	0.00	-	0.50	0.14	0.07	0.23

Figure 6. ERP waves for the four conditions (negative values plotted upwards) (A), P3 amplitude for participants with and without SAD (B), and results of the heritability analysis (C). P3 was computed as an area measure in the time window from 360 to 500 ms at electrode Pz. Heritability results did not remain significant after correction for performing multiple tests.

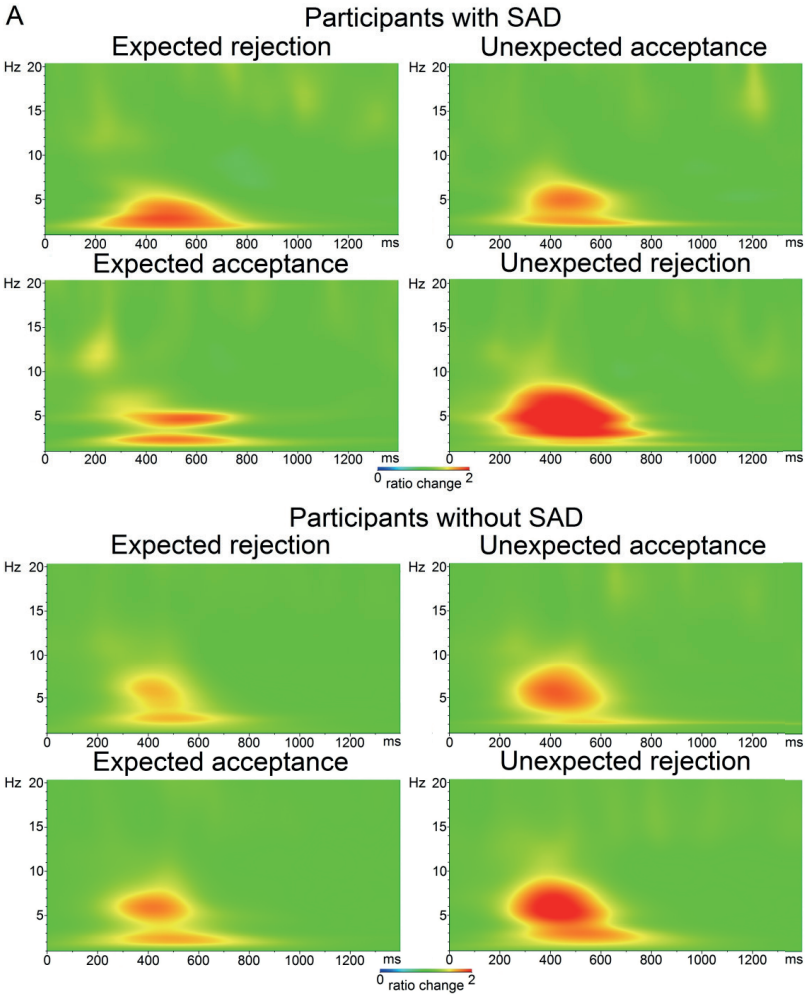
Note: SAD = social anxiety disorder; NoNo = expected rejection; NoYes = unexpected acceptance; YesYes = expected acceptance; YesNo = unexpected rejection; h^2 = heritability; SE = standard error. SE could not be computed if $h^2 = 0$.

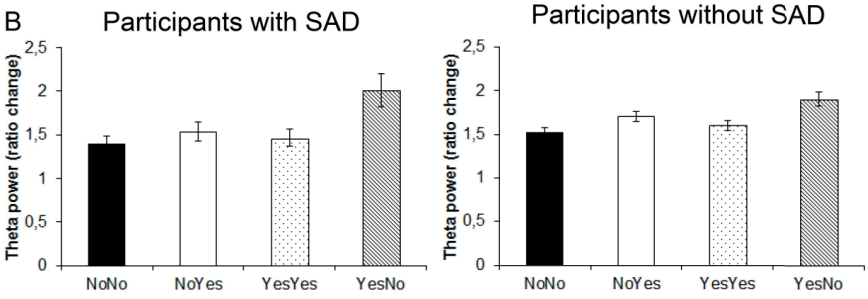
Neural oscillatory power

Theta. There was no co-segregation within families between SAD and theta power across conditions (Figure 7 and Supplementary table 3). The two-way interaction between congruency and valence, $\beta = 0.17$, $p < 0.001$, revealed that theta power was increased after unexpected rejection feedback compared to the other conditions. This effect was increased in SAD, as indicated by the three-way interaction between SAD, congruency and feedback, $\beta = 0.15$, $p < 0.001$. Heritability analyses showed that theta power was not heritable, the heritability estimate of theta after expected acceptance feedback was only significant if we did not correct for multiple tests, $h^2 = 0.42$, $p = 0.03$.

Age and sex

Most variables (except P3 and theta) showed linear effect of age, and a quadratic effect of age (except bias score and VAS ratings). Most variables became stronger with increasing age and showed a peak between 20-40 years. Only the FRN became less strong with increasing age. There was no effect of sex on any of the behavioral, ERP or neural oscillatory findings.





C Heritability analysis

	h^2	SE	p	p (Age)	p (Age^2)	p (Sex)
NoNo	0.03	0.11	0.38	0.12	0.10	0.93
NoYes	0.11	0.17	0.23	0.29	0.002	0.002
YesYes	0.42	0.25	0.03	0.01	0.39	0.27
YesNo	0.35	0.27	0.07	0.84	< 0.001	0.73
YesNo vs Rest	0.00	-	0.50	0.24	0.01	0.60

Figure 7. Time-frequency plots for the four conditions (A), theta ratio change for participants with and without SAD (B), and results of the heritability analysis (C). Theta ratio change was computed within a time window from 300 to 500 ms at electrode Fz. Heritability results did not remain significant after correction for performing multiple tests.

Note: SAD = social anxiety disorder; NoNo = expected rejection; NoYes = unexpected acceptance; YesYes = expected acceptance; YesNo = unexpected rejection; h^2 = heritability; SE = standard error. SE could not be computed if $h^2 = 0$.

Discussion

The goal of this study was to investigate whether behavioral and electrocortical responses to social evaluation are candidate endophenotypes of SAD. Using a validated paradigm (SJP) and a unique two-generation family design we tested two criteria for endophenotypes: co-segregation with SAD within families and heritability. Results revealed that participants with SAD expected rejection more often before and during the SJP, and had experienced rejection more often after the SJP. Reaction time associated with indicating their expectations during the SJP was longer in participants with SAD compared to participants without SAD. Electrocortical results revealed that increased N1 in response to all conditions, except after expected acceptance feedback, co-segregated with SAD. Increased FRN after unexpected rejection feedback compared to the other conditions co-segregated with SAD. P3 in response to acceptance versus rejection feedback co-segregated with SAD. Finally, increased theta power after unexpected rejection feedback compared to the other conditions co-segregated with SAD. The heritability estimates were not significant if we corrected for multiple tests. However, if we did not apply this correction, reaction time for acceptance expectations, N1 in response to expected rejection, and P3 in response to acceptance versus rejection feedback would be heritable.

As predicted, participants with SAD expected rejection more often before and during the SJP, and had experienced rejection more often after the SJP than participants without SAD. In general, people show an optimism bias in the SJP; they expect to be accepted more than rejected (Dekkers et al., 2015; Gunther Moor, Crone, et al., 2010; Van der Molen et al., 2017; Van der Molen et al., 2014; Van der Veen et al., 2016). The currently observed pessimism bias in SAD corroborates behavioral findings on the Island Getaway task (Cao et al., 2015), and cognitive-behavioral findings suggesting that patients with SAD predict future social events more negatively (Hirsch & Clark, 2004). This is an important focus of cognitive-behavioral therapy in SAD (Heimberg, 2002). In addition, the reaction time associated with

indicating expectations co-segregated with SAD, extending findings on fear of negative evaluation in healthy females (Van der Molen et al., 2014). This might suggest that participants with SAD are less certain about their choices, or show more self-focused attention and vigilance during the SJP (Van der Molen et al., 2014). The heritability estimate for reaction time associated with indicating acceptance-expectations was only significant if we did not correct for multiple tests, suggesting that this might be a candidate behavioral endophenotype of SAD. This could mean that the other behavioral measures are symptoms of SAD instead of mechanisms underlying the development of SAD. Together, these behavioral findings showed that the SJP is a useful task to measure responses to social evaluation in SAD.

Patients with SAD showed an increased N1 in all conditions of the SJP, except after expected acceptance feedback. Although we had no a priori predictions regarding this component in the SJP, this finding is in accord with cognitive-behavioral studies showing hypervigilance to socially threatening stimuli (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013), and ERP studies showing increased early attentional ERPs in SAD (Harrewijn et al., 2017; Staugaard, 2010). Indeed, the N1 is related to attention (Luck, 2005; Luck & Kappenman, 2013), and increased for emotional compared to neutral stimuli (Hajcak et al., 2013). So, the N1 might reflect an early attentional bias towards socially threatening stimuli. The N1 in our study was not increased after expected acceptance feedback, probably because this condition is the least threatening. The heritability estimate of N1 in response to expected rejection feedback was only significant if we did not correct for multiple tests. Thus, N1 after expected rejection feedback might be a candidate endophenotype of SAD. Our study was the first to show that the N1 is an important component to study in the SJP and might be a candidate endophenotype of SAD.

Participants with SAD showed an increased FRN after unexpected rejection feedback compared to the other conditions. This finding shows that both congruency and valence of social feedback modulate the FRN in social anxiety, whereas in healthy participants the FRN is only sensitive to congruency in the SJP (Dekkers et al., 2015; Van der Molen et al., 2017; Van der Molen et al., 2014). Increased FRN after unexpected rejection feedback in SAD might reflect that the usual FRN response to incongruent feedback is intensified by a selective bias for negative evaluation (Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004). We observed a similar effect in theta power: increased theta power after unexpected rejection feedback co-segregated with SAD. This result corroborates previous

findings that theta power is increased after unexpected rejection feedback in the SJP (Van der Molen et al., 2017). Some have suggested that theta power is related to processing social threat (Cristofori et al., 2013; Van Noordt, White, et al., 2015). This would indicate that unexpected rejection feedback is the most threatening condition in the SJP, a notion that is substantiated by heart rate studies using the SJP (Dekkers et al., 2015; Gunther Moor, Bos, Crone, & Van der Molen, 2014; Gunther Moor, Crone, et al., 2010; Van der Veen et al., 2014). Here we demonstrate that this effect is exaggerated in SAD, suggesting that unexpected rejection feedback is even more threatening for participants with SAD. This is in line with cognitive-behavioral studies showing that patients with SAD interpret mildly negative social events in a catastrophic way (Clark & McManus, 2002), and show extreme fear of negative evaluation (APA, 2013; Rapee & Heimberg, 1997). Interestingly, in SAD, both phase-locked (FRN) and induced oscillatory power (theta) are modulated by congruency and valence of social evaluative feedback. Cavanagh and Shackman (2015) argue that both the FRN and theta power are generated by the midcingulate cortex and might signal the need for adaptive control in uncertain situations. Receiving unexpected rejection feedback might reflect such a situation because there is uncertainty about the optimal course of action. Together these findings show that FRN and theta power are promising electrocortical markers of SAD, but did not meet the criteria of endophenotypes because they were not heritable. This might suggest that FRN and theta power are more influenced by environmental factors. Indeed, previous studies have found that neural correlates in response to rejection are related to environmental factors such as chronic rejection during childhood (Will, Van Lier, Crone, & Guroglu, 2016), time spent with friends (Masten, Telzer, Fuligni, Lieberman, & Eisenberger, 2012), attachment (White, Wu, Borelli, Mayes, & Crowley, 2013; White et al., 2012), early separation experiences (Puetz et al., 2014), and maltreatment (Puetz et al., 2016).

The P3 was larger for acceptance than rejection feedback, regardless of feedback congruency, in participants with SAD, but not in participants without SAD. This is in line with the interpretation of the P3 as an index of processing emotionally motivational stimuli (Hajcak et al., 2013). This would suggest that acceptance feedback is even more important for participants with SAD than participants without SAD, which is in line with cognitive theories emphasizing the importance of positive social evaluation for patients with SAD (Rapee & Heimberg, 1997). The P3 might also reflect reward processing (Van der Veen et al., 2016; Van der Veen et al., 2014), but this interpretation was based on an increased P3 only after expected acceptance. Our P3 results might be explained by subjective probability (Ferdinand et al., 2012; Johnson, 1986). That is, participants with SAD probably expect less acceptance

by peers and due to this low subjective probability of acceptance feedback, P3 amplitudes in response to acceptance feedback are increased. This is supported by the behavioral data showing that participants with SAD expected to be accepted less often, and needed more time for indicating their expectations than participants without SAD. Heritability estimates of P3 in response to expected and unexpected acceptance feedback were only significant if we did not correct for multiple tests, suggesting that this might be a candidate endophenotype of SAD.

This unique two-generation family design has given us the opportunity to study two endophenotype criteria: co-segregation with SAD within families and heritability. Since this is the first study administering the SJP in participants with SAD, our findings should be confirmed in future research. Future research should also focus on specificity of these measures for SAD. A few limitations should be taken into account. First, since only few non-target family members were diagnosed with SAD, we could not calculate the genetic correlation between SAD and the candidate endophenotypes. Second, none of the heritability estimates survived corrections for performing multiple tests. Thus, although we found interesting results on behavioral and electrocortical responses to social evaluation in SAD, the robustness of these effects should be validated. Third, gene-environment interactions could also have played a role in these results, since we were not able to correct for shared environmental effects. Fourth, participants with and without SAD showed a different number of artifact-free trials, which is inherent to the behavioral finding that participants with SAD expected rejection more often. Fifth, the degree to which the currently reported endophenotypes are specific to SAD remains uncertain, particularly due to the co-occurrence of depressive symptoms in participants with SAD. Since SAD and depression are overlapping constructs (Cerda, Sagdeo, Johnson, & Galea, 2010; Hettema, Chen, Sun, & Brown, 2015; Mineka, Watson, & Clark, 1998), controlling for depressive symptoms would have been invalid. That is, controlling for depression would remove important variance relevant to SAD (Miller & Chapman, 2001), as participants with SAD reported significantly higher levels of depressive symptoms than those without SAD. It should be noted, however, that the patients in the current study had SAD as primary diagnosis, and did not have a current depressive episode. Moreover, a recent study using the same paradigm has shown that the behavioral and electrophysiological responses to social evaluation were not related to depressive symptoms in healthy adults (Van der Veen et al., 2016). We acknowledge that specificity of these candidate endophenotypes should be an important focus in future studies. One approach would be to compare patients with SAD with and without comorbid depression. Another approach would be to cross syndrome boundaries and focus on traits shared across disorders

(Levy & Ebstein, 2009). Such future endeavors would be of critical importance to determine specificity of these endophenotypes, or whether they could instead be conceptualized as transdiagnostic markers that will aid in understanding the etiology of psychopathology.

To conclude, in the present study reaction time for indicating acceptance-expectations might be a candidate behavioral endophenotype of SAD, possibly reflecting increased uncertainty or self-focused attention and vigilance during social evaluation. At the electrocortical level, this vigilance seems tracked by an increased N1 after rejection feedback and unexpected acceptance. At later processing stages, we observed increased P3 amplitudes to acceptance feedback as endophenotype of SAD. These behavioral and electrocortical endophenotypes might provide insight in genetic mechanisms underlying SAD (Cannon & Keller, 2006; Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013). Future research should validate these findings, and investigate whether training these attentional biases might prevent the development of SAD in persons with a genetic vulnerability. Another interesting venue for future research is investigating how parents might influence this hypervigilance for socially threatening stimuli and/or focus on positive feedback in their children with a genetic vulnerability for SAD.

Supplementary table 1

Number of artifact-free trials per condition for participants with and without SAD.

Condition	Participants	Mean	Standard deviation	Minimum	Maximum
Expected rejection	With SAD	42.50	9.57	21	60
	Without SAD	33.43	8.45	10	52
Unexpected acceptance	With SAD	38.28	11.79	21	63
	Without SAD	30.24	9.32	10	60
Expected acceptance	With SAD	32.72	12.23	7	51
	Without SAD	37.87	11.58	7	58
Unexpected rejection	With SAD	27.94	10.62	7	52
	Without SAD	34.79	9.63	10	64

Note: Two participants had less than 10 clean trials in the 'expected acceptance' and 'unexpected rejection' conditions. We decided not to exclude these participants, because this was probably related to a negative expectation bias (which is related to SAD) as they had far more clean 'expected rejection' and 'unexpected acceptance' trials (more than 51). When we excluded these two participants, the results remained largely the same. Only, for the P3 the three-way interaction between SAD, congruency and feedback was significant, $\beta = 0.50$, $p = 0.002$, while this effect was marginally significant in the original analysis. Furthermore, heritability estimates of N1 during expected rejection feedback ($h^2 = 0.24$, $p = 0.08$, was significant in the original analysis) and of theta power during unexpected rejection feedback ($h^2 = 0.40$, $p = 0.04$, was marginally significant in the original analysis) changed.

SAD = social anxiety disorder

Supplementary table 2

Outcomes of the standardized diagnostic interview for participants with and without SAD separately.

		Participants with SAD (12 females, 6 males)	Participants without SAD (47 females, 50 males)
Depressive episode	Current	0	1
	Past	7	15
Dysthemia	Past	1	1
Bipolair 2 disorder	Current	0	0
	Past	0	0
Panic disorder	Current	2	0
	Lifetime	3	3
Agoraphobia	Current	5	2
	Past	0	1
Seperation anxiety	Current	0	1
Specific phobia		1	3
Obsessive-compulsive disorder	Current	1	0
Post-traumatic stress disorder	Current	0	0
Generalized anxiety disorder	Current	2	0

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

SAD = social anxiety disorder

Supplementary table 3

Outcomes of the co-segregation analyses for (A) behavior (bias score, VAS ratings, reaction time), (B) ERPs (N1, FRN, P3), and (C) neural oscillatory power (theta). Significant effects are underlined.

A Behavioral data			
Dependent variable: bias score			
	β	*SE*	*p*
Intercept	53.29	3.84	< 0.001
SAD	-14.39	2.98	< 0.001
Age	4.65	1.12	< 0.001
Age^2	-1.38	1.39	0.32
Sex	1.30	2.13	0.54
Dependent variable: VAS ratings			
	β	*SE*	*p*
Intercept	79.49	4.19	< 0.001
SAD	-12.05	5.94	0.04
Condition (before vs after SJP)	-12.88	1.38	< 0.001
SAD*Condition	1.97	3.48	0.57
Age	3.61	1.03	< 0.001
Age^2	-2.16	1.41	0.13
Sex	-0.97	1.96	0.62
Dependent variable: reaction time			
	β	*SE*	*p*
Intercept	1651.39	95.40	< 0.001
SAD	260.66	98.47	0.01
Condition (acceptance vs rejection)	31.25	16.09	0.05
SAD*Condition	-135.78	40.67	< 0.001
Age	79.06	28.18	0.01
Age^2	-72.56	34.54	0.04
Sex	-40.69	51.24	0.43

B ERP data			
Dependent variable: N1 at FCz			
	β	*SE*	*p*
Intercept	-2.00	0.53	< 0.001
SAD	-1.24	0.49	0.01
Congruency	-0.42	0.24	0.07
Valence	-0.13	0.21	0.55
Congruency*Valence	0.08	0.15	0.60
SAD*Congruency	0.76	0.42	0.07
SAD*Valence	1.03	0.43	0.02
SAD*Congruency*Valence	0.52	0.15	< 0.001
Age	-0.54	0.15	< 0.001
Age^2	0.76	0.18	< 0.001
Sex	0.18	0.29	0.52
Dependent variable: FRN at Fz			
	β	*SE*	*p*
Intercept	-1.33	0.93	0.15
SAD	0.65	0.83	0.44
Congruency	-0.32	0.37	0.40
Valence	0.49	0.37	0.18
Congruency*Valence	0.60	0.27	0.03
SAD*Congruency	0.36	0.62	0.56
SAD*Valence	-1.01	0.64	0.11
SAD*Congruency*Valence	-0.94	0.27	< 0.001
Age	2.13	0.24	< 0.001
Age^2	-1.65	0.29	< 0.001
Sex	-0.67	0.49	0.18
Dependent variable: P3 at Pz			
	β	*SE*	*p*
Intercept	9.11	1.22	< 0.001
SAD	-0.64	0.93	0.49
Congruency	-0.94	0.29	0.001
Valence	-0.68	0.26	0.01

Congruency*Valence	1.34	0.19	< 0.001
SAD*Congruency	0.04	0.48	0.94
SAD*Valence	-1.02	0.47	0.03
SAD*Congruency*Valence	0.34	0.19	0.07
Age	0.02	0.34	0.95
Age^2	-0.97	0.41	0.02
Sex	0.07	0.65	0.91
C Neural oscillatory power			
Dependent variable: Theta at Fz			
	β	*SE*	*p*
Intercept	1.87	0.14	< 0.001
SAD	-0.17	0.11	0.11
Congruency	0.08	0.06	0.20
Valence	-0.09	0.05	0.10
Congruency*Valence	0.17	0.04	< 0.001
SAD*Congruency	0.10	0.13	0.45
SAD*Valence	0.13	0.10	0.19
SAD*Congruency*Valence	0.15	0.04	< 0.001
Age	-0.05	0.04	0.16
Age^2	-0.09	0.05	0.049
Sex	-0.11	0.07	0.14

Note: SE = standard error; SAD = social anxiety disorder; VAS = visual analogue scale; ERP = event-related potential; Congruceny = expected versus unexpected; Valence = acceptance versus rejection; FRN = feedback-related negativity.

Chapter 7

Summary and general discussion

Summary

The goal of this dissertation was to delineate psychophysiological endophenotypes of social anxiety disorder (SAD). Studying endophenotypes could be seen as a first step in unraveling genetic mechanisms underlying psychiatric disorders, because endophenotypes are supposedly influenced by less genes than complex psychiatric disorders (Cannon & Keller, 2006; Glahn et al., 2007). In addition, endophenotypes could yield better understanding of the biological mechanisms underlying SAD (Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013). Our Leiden Family Lab study on SAD focused on two criteria for endophenotypes: co-segregation with SAD within families and heritability. Patients with SAD participated with their partner and children, as well as their siblings with partner and children. This dissertation focused on EEG and heart rate measures during resting state, a social performance task and a social judgment paradigm. In the social performance task, participants watched and evaluated a speech of a female peer and then gave a speech in front of a video camera themselves. In the social judgment paradigm, participants received social acceptance or rejection feedback, after indicating their expectations about the upcoming feedback.

The *second chapter* gives an overview of the most frequently studied EEG measures of information processing biases in SAD. Studies on EEG spectral characteristics have shown that delta-beta correlation during anticipation of and recovery from a stressful social situation is a promising electrocortical marker, possibly reflecting the alleged imbalance between cortical and subcortical brain regions (Bishop, 2007; Bruhl et al., 2014; Cremers, Veer, Spinhoven, Rombouts, Yarkoni, et al., 2015; Miskovic & Schmidt, 2012). The event-related potential studies have shown information processing biases during early processing of faces (P1) and errors (error-related negativity; ERN). Increased P1 amplitude in response to faces possibly reflects hypervigilance to threatening stimuli (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013), and increased ERN amplitude possibly reflects increased self-focused attention (Bögels & Mansell, 2004; Clark & McManus, 2002) or perfectionism (Clark & Wells, 1995).

The *third chapter* reports on the validation of our newly developed social performance task in high and low socially anxious females. High socially anxious females reported more nervousness and avoidance during the social performance task than low socially anxious females. We jointly examined frontal alpha asymmetry and delta-beta correlation in this task, and found that only negative delta-beta correlation during anticipation of and recovery from this stressful social situation was related to social anxiety. Increased negative delta-beta

correlation is interpreted to reflect the imbalance between cortical and subcortical regions as found in fMRI studies in SAD (Bruhl et al., 2014; Cremers, Veer, Spinhoven, Rombouts, Yarkoni, et al., 2015; Miskovic & Schmidt, 2012) or in anxiety more general (Bishop, 2007).

In the *fourth chapter* we investigate whether delta-beta correlation could also be seen as an endophenotype of SAD. We found that delta-low beta correlation co-segregated with (sub)clinical SAD within families and was heritable. So, delta-low beta correlation meets the second and third criteria for endophenotypes, and thus might be an endophenotype of SAD.

The *fifth chapter* focuses on heart rate variability during resting state and the social performance task as a candidate endophenotype of SAD. Heart rate variability did not co-segregate with SAD within families, but was heritable. So, heart rate variability did not meet the second criterion for endophenotypes. We suggest that heart rate variability might reflect a transdiagnostic genetic vulnerability for internalizing disorders, related to reduced flexibility due to impaired inhibition (Chalmers et al., 2014; Thayer & Lane, 2000) or generalized unsafety (Brosschot et al., 2016).

The *sixth chapter* describes whether behavioral and EEG measures in the social judgment paradigm could be seen as endophenotypes of SAD. Reaction time for acceptance-expectations, N1 amplitude in response to expected rejection feedback, and P3 amplitude in response to acceptance feedback met the two criteria for endophenotyps that we assessed (co-segregation and heritability). Reaction time for acceptance-expectations possibly reflects increased uncertainty or self-focused attention and vigilance during the social judgment paradigm (Van der Molen et al., 2014). Increased N1 amplitude possibly reflects hypervigilance to socially threatening stimuli (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013), and increased P3 amplitude might reflect that positive feedback is more important for, and/or less expected by, participants with SAD (Ferdinand et al., 2012; Johnson, 1986; Rapee & Heimberg, 1997). Feedback-related negativity (FRN) and theta power were increased after unexpected rejection feedback compared to the other conditions in patients with SAD, but these measures were not heritable.

General discussion

This dissertation focused on three criteria for endophenotypes: association with SAD (*second chapter*), co-segregation with SAD within families, and heritability (*fourth, fifth* and *sixth chapter*). Delta-beta correlation, P1 and ERN meet the first criterion for endophenotypes. Delta-low beta correlation, reaction time, N1, and P3 meet the second and third criteria for endophenotypes. We have not assessed the fourth (non-affected versus general population) and fifth (state-independence) criteria for endophenotypes, so we have to be careful with concluding that these psychophysiological measures are endophenotypes of SAD. However, delta-beta correlation did not differ in patients with SAD between two visits approximately one week apart (Miskovic, Moscovitch, et al., 2011), which suggests stability over time and could be linked to the fifth criterion for endophenotypes (state-independence). Some studies have shown moderate to strong test-retest reliability for P1, N1, P3 and ERN amplitudes, albeit in different paradigms than employed in this dissertation (Cassidy, Robertson, & O'Connell, 2012; Hall et al., 2006; Polich, 2007; Weinberg & Hajcak, 2011). However, if the fifth criterion is interpreted as clearly distinctive states such as in bipolar disorder, this would not be applicable to SAD. To conclude, delta-beta correlation, N1 and P3 are candidate endophenotypes of SAD and future research should also study the other criteria for endophenotypes.

The results described in this dissertation also show which psychophysiological measures give less insight in the underlying mechanisms of SAD. First, frontal alpha asymmetry seems to be related to social anxiety or behavioral inhibition in children (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Henderson et al., 2001; Henderson et al., 2004), but the findings in adults are mixed (see *chapter two*). Some early studies have found an effect of social anxiety on frontal alpha asymmetry (Davidson et al., 2000; Schmidt, Fox, Schulkin, & Gold, 1999), but more recent studies have only found an effect in specific conditions (Cole et al., 2012) or not at all (Beaton et al., 2008; Harrewijn et al., 2016). Also, when presenting the findings of this dissertation and talking to other researchers, it became clear that there are unpublished null findings on frontal alpha asymmetry as well. Inconsistencies in findings on frontal alpha asymmetry and SAD might be related to the role of comorbid depression (Thibodeau et al., 2006). Second, previous studies have reported mixed findings on heart rate variability in SAD (see *chapter five*). Decreased heart rate variability during resting state is related to different internalizing disorders, such as several anxiety disorders (Chalmers et al., 2014; B. H. Friedman, 2007; Pittig et al., 2013), and

depression (Kemp et al., 2012; Kemp et al., 2010). Therefore, we suggest in *chapter five* that heart rate variability is a transdiagnostic genetic vulnerability for internalizing disorders.

This dissertation aimed at delineating psychophysiological endophenotypes of SAD to gain more insight in the mechanisms underlying the development and maintenance of SAD. Information processing biases play an important role in the development and maintenance of SAD (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013; Wong & Rapee, 2016), and the candidate endophenotypes in this dissertation might be reflective of these information processing biases. For example, early event-related potentials might be reflective of an early attention bias to socially threatening stimuli, and delta-beta correlation might be reflective of an interpretation bias during anticipation (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013). Of course, future research is necessary to link these psychophysiological endophenotypes to information processing biases, and to use these psychophysiological endophenotypes in treatment to alleviate SAD symptoms. In the following paragraphs, I will discuss directions for future research and the clinical implications of research on psychophysiological endophenotypes.

Directions for future research

Studying endophenotypes could be seen as a first step in unraveling genetic mechanisms underlying psychiatric disorders, because endophenotypes are proposed to be related to fewer genes than complex psychiatric disorders such as SAD (Cannon & Keller, 2006; Glahn et al., 2007). Future research should investigate the genes that are related to psychophysiological endophenotypes, and whether these genes are also related to SAD. This use of endophenotypes has been criticized in recent studies, as endophenotypes are also influenced by many genes (Flint et al., 2014; Iacono et al., 2016). Nevertheless, endophenotypes could yield a better understanding in the biological mechanisms underlying SAD (Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013) and could help in interpreting genetic findings (De Geus, 2010; Flint et al., 2014). Below, I discuss how psychophysiological endophenotypes of SAD might be used in future studies to provide more insight on information processing biases and their role in the maintenance and development of SAD.

So far, research has focused on psychophysiological measures of separate information processing biases. However, it would be interesting to investigate how these psychophysiological measures influence each other. For example, it might be possible that

hypervigilance (as reflected in early event-related potentials) influences later processing of socially threatening stimuli (as reflected in later event-related potentials). EEG is the ideal method to study this, because of the high temporal resolution (Amodio et al., 2014; M. X. Cohen, 2011; Ibanez et al., 2012; Luck, 2005). Moreover, it is proposed that these information processing biases in SAD have formed a persistent cycle: they are triggered by social situations, repeated within the situation, and carried forward over time during anticipation (Clark & McManus, 2002; Morrison & Heimberg, 2013). This persistent cycle plays an important role in the maintenance of SAD, but has only scarcely been studied. Few studies have focused on the influence of anticipation on later processing in healthy participants. Anticipation of public speaking enhanced early processing of negative faces (Wieser et al., 2010). However, anticipation of receiving social evaluative feedback (as measured by the stimulus preceding negativity) did not influence processing of this feedback as measured by the FRN and P3 (Van der Molen et al., 2014). This is an interesting line of research to continue, as anticipation might have an increased effect in patients with SAD.

Another important area for future research is investigating the link between psychophysiological endophenotypes and behavior. A promising line of research on the ERN has focused on post-error slowing, which is the tendency of people to respond slower after they have made an error on the previous trial (Danielmeier & Ullsperger, 2011; Gehring & Fencsik, 2001). Only one study has investigated this in SAD and found no difference between participants with and without SAD (Endrass et al., 2014), but this should be confirmed in future studies. Extending this line of research to other candidate psychophysiological endophenotypes might yield promising information. For example, it should be studied how patients with SAD interact with people, after the hypervigilant reaction to faces. They might try to use safety behaviors, such as avoiding eye contact, to hide their extreme initial reaction towards this person (Clark & Wells, 1995; Wells et al., 1995). Furthermore, it should also be studied how decreased delta-beta correlation during anticipation of a stressful social situation influences subsequent behavior. Most participants were nervous during our social performance task, but it might be the case that nervous behavior during the speech is most apparent in participants with increased delta-beta correlation during anticipation. The relation between psychophysiological endophenotypes and behavior might play an important role in the maintenance of SAD.

Psychophysiological endophenotypes of SAD might reflect a genetic vulnerability for developing SAD. However, not everyone with this genetic vulnerability will eventually develop the disorder. An important next step is to study which persons with this genetic

vulnerability will develop SAD, and which persons will not. Many factors play a role in the development of SAD, such as temperament, cognitive factors, peer relationships, parenting, adverse life events and cultural variables (Spence & Rapee, 2016). It should be investigated how these factors interact with endophenotypes. Gender should also be taken into account in future studies, as there might be gender differences in psychophysiological endophenotypes or factors interacting with these endophenotypes. Besides studying which factors increase the risk for developing SAD in persons with a certain endophenotype, future longitudinal studies should also investigate which factors protect persons with a certain endophenotype from developing SAD.

Future longitudinal studies should also investigate how these psychophysiological endophenotypes develop over time. As described in *chapter two*, only the ERN has been studied in both adults and children. The other psychophysiological endophenotypes could also be compared between adults and children, preferably in a longitudinal design. Furthermore, endophenotypes might affect the development of SAD differently across the lifespan. For example, Haller et al. (2014) propose that normal development in brain regions associated with emotional and social processes increases information processing biases in attention, interpretation and expectations. This puts adolescents at increased risk for developing SAD (Haller et al., 2014). Furthermore, adolescents become more focused on peers instead of their parents (Blakemore & Mills, 2014; R. W. Larson & Richards, 1991; R. W. Larson, Richards, Moneta, Holmbeck, & Duckett, 1996), and react more intensely to social rejection (Gunther Moor et al., 2014; Sebastian, Viding, Williams, & Blakemore, 2010). So, normal development might enhance information processing biases, which might lead to SAD in certain adolescents.

Clinical implications

The ultimate goal of this research on psychophysiological endophenotypes of SAD is to gain more insight in the underlying mechanisms of this disorder, and to eventually improve early detection and intervention. These insights can be used to focus treatment on the most important underlying mechanisms of SAD. For example, if it turns out that aberrant early processing of socially threatening stimuli is most important for maintaining SAD, it might help to focus treatment on alleviating this initial reaction. Furthermore, endophenotypes might eventually predict which treatment works best for which patient, as not all treatments might be equally effective for all patients with SAD. For example, patients with

psychophysiological endophenotypes related to attention biases (such as early event-related potentials) might need a different treatment than patients with psychophysiological endophenotypes related to interpretation biases (such as delta-beta correlation during anticipation). Although EEG research on predicting treatment response in anxiety disorders is only in its infancy (Lueken et al., 2016), some recent studies have found promising results. That is, P1 amplitude in response to faces might be a predictor of treatment outcome and N2 and LPP amplitudes in response to faces might be predictors of treatment response in anxiety disorders (Bunford et al., 2017; Hum et al., 2013).

Second, endophenotypes might play a role in early detection of SAD and the development of preventive interventions. If we could assess delta-beta correlation during anticipation, or N1/P3 amplitudes in the social judgment paradigm in children before they have developed SAD, it might be easier to intervene. Interestingly, children with a parent with SAD showed increased delta-beta correlation during resting state, compared to children without a parent with SAD (Miskovic, Campbell, et al., 2011). Insight in the factors that influence the development of SAD in children with a genetic vulnerability will help in the development of preventive interventions. Endophenotype research is not only useful for clinical settings, but might even be useful for school settings. School is a very stressful environment for children with SAD, due to the many interactions with peers and teachers. It should be investigated how teachers could encourage children with a genetic vulnerability for SAD to interact with peers and to gain positive experiences.

Conclusion

The goal of this dissertation was to delineate psychophysiological endophenotypes of SAD. Delta-beta correlation during anticipation of a stressful social situation, and N1 and P3 amplitude in the social judgment paradigm are candidate endophenotypes of SAD. Future research should continue this promising line of endophenotype research in three different directions. First, it should be investigated how these endophenotypes maintain SAD by studying their influence on later processing stages and subsequent behavior. Second, it should be studied which factors influence the development of SAD in persons with this genetic vulnerability and how endophenotypes develop over time. Third, it should be investigated how the endophenotypes could be best used in treatment, for example by giving more insight in the patho-etiology of SAD, and by improving early detection and preventive interventions.

Socially anxious parents were motivated to participate in our family study because they recognized their own anxiety in their children and did not want their children to develop the same problems as they were having. In addition, many participants came from the other end of the country and had to drive three hours (with young children on the back seat) to participate in our study. This nicely illustrates how motivated these family members were to do something for their relative with social anxiety disorder. These examples underline the importance of research on SAD and the need for future studies on factors that might help alleviating SAD.

Chapter 8

Nederlandse samenvatting

Verlegen ouder, verlegen kind?

Onderzoeken van psychofysiologische endofenotypes van sociale angststoornis

Eén van de deelnemers in het Leidse familie-onderzoek over sociale angststoornis vertelde me dat ze alledaagse dingen, zoals naar de kapper gaan, het liefst doet in het volgende dorp, in plaats van in het dorp waar ze woont. Ze doet dit omdat ze niet weet hoe ze moet reageren als ze onverwachts een bekende tegenkomt: Moet ze een praatje maken? Moet ze gewoon doorlopen? Ze weet niet hoe ze met deze sociale situatie moet omgaan en vermijdt daarom de situatie helemaal.

Natuurlijk voelt iedereen zich weleens sociaal angstig of verlegen. Rapee en Spence (2004) stellen dat sociale angst als een continue schaal gezien kan worden: aan de ene kant mensen die helemaal geen angst hebben in sociale situaties, aan de andere kant mensen met sociale angststoornis. Mensen met sociale angststoornis tonen extreme angst in sociale situaties en proberen deze situaties zo veel mogelijk te vermijden (APA, 2013). Deze angst duurt meer dan zes maanden en beïnvloedt het dagelijks leven sterk. Mensen met sociale angststoornis missen bijvoorbeeld kansen op het werk, omdat ze zich niet durven te laten zien. Mensen met sociale angststoornis laten afwijkingen zien in de informatieverwerking. Zij focussen bijvoorbeeld hun aandacht op bedreigende stimuli, interpreteren sociale situaties negatiever dan andere mensen, onthouden vooral de slechte dingen uit sociale situaties en stellen zich voor hoe ze slecht reageren in sociale situaties (Bögels & Mansell, 2004; Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004; Morrison & Heimberg, 2013; Wong & Rapee, 2016). Het is belangrijk om meer te weten te komen over deze onderliggende mechanismes van sociale angststoornis, om de stoornis vroeg te kunnen detecteren en patiënten met sociale angststoornis beter te kunnen behandelen.

Eén manier om deze afwijkingen in de informatieverwerking te kunnen meten is via psychofysiologische maten zoals EEG en hartslag. EEG geeft informatie over wanneer het brein informatie verwerkt. In dit proefschrift heb ik mij gefocust op psychofysiologische maten om meer te weten te komen over de mechanismes onderliggend aan sociale angststoornis. Ik heb mij specifiek gericht op frontale alpa asymmetry, delta-beta correlatie en hartslagvariabiliteit tijdens rust en het anticiperen of bijkomen van een stressvolle sociale situatie. Daarnaast heb ik mij gericht op verschillende EEG componenten (N1, feedback-related negativity, P3 en theta) in reactie op sociale evaluatie. Het doel van dit proefschrift

was om te onderzoeken of deze psychofysiologische maten ook endofenotypes van sociale angststoornis zijn.

Endofenotypes

Dit is een veelbelovende richting in psychiatrisch onderzoek, gebaseerd op het idee dat sommige psychiatrische stoornissen erfelijk zijn. Het is heel moeilijk om de genen te vinden van complexe psychiatrische stoornissen, omdat er zoveel genen betrokken zijn en omdat verschillende patiënten dezelfde stoornis op andere manieren kunnen uiten. Endofenotype-onderzoek zet een stap 'tussen' de genen en de psychiatrische stoornis. Endofenotypes zijn kenmerken (bijv. hersenactiviteit, gedrag of vragenlijsten) die gerelateerd zijn aan de psychiatrische stoornis en erfelijk zijn. Deze endofenotypes zijn specifieker dan een complexe stoornis en het idee is dat ze daarom beïnvloed worden door minder genen. Daarnaast kunnen endofenotypes inzicht bieden in de onderliggende mechanismen van psychiatrische stoornissen (Cannon & Keller, 2006; Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013).

De erfelijkheid van sociale angststoornis wordt geschat tussen de 20 en 56 procent (Distel et al., 2008; Isomura et al., 2015; Kendler et al., 1992; Middeldorp et al., 2005; Nelson et al., 2000), maar nog geen enkele studie heeft endofenotypes van sociale angststoornis onderzocht. Het doel van dit proefschrift was daarom het onderzoeken van psychofysiologische endophenotypes van sociale angststoornis. Een psychofysiologische maat moet aan verschillende criteria voldoen om gezien te kunnen worden als een endofenotype (Glahn et al., 2007; Gottesman & Gould, 2003):

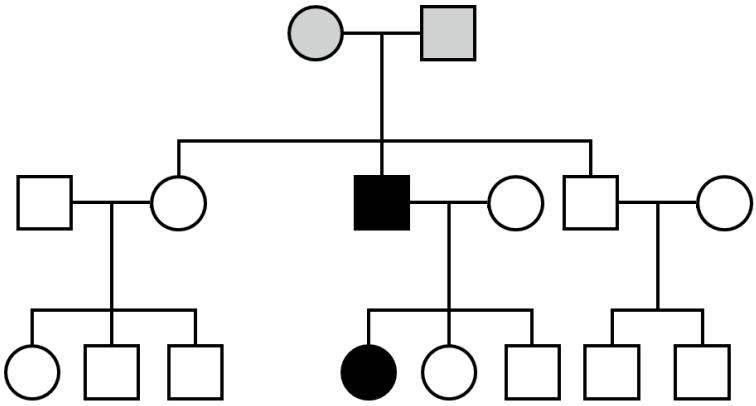
1) De maat moet gerelateerd zijn aan de stoornis.
2) De maat en de stoornis moeten samengaan binnen families (mensen met de stoornis moeten ook de maat laten zien).
3) De maat moet erfelijk zijn.
4) Familieleden die geen last van de stoornis hebben, moeten de maat meer laten zien dan mensen uit de 'normale populatie'.
5) De maat moet onafhankelijk zijn van staat.

Het eerste criterium is al voor verschillende psychofysiologische maten onderzocht. Dit is gedaan in studies waarin EEG maten worden vergeleken tussen mensen met en zonder sociale angststoornis (Miskovic & Schmidt, 2012; Schulz et al., 2013; Staugaard, 2010). In dit proefschrift onderzocht ik het tweede en derde criterium, door mensen met sociale angststoornis en hun familieleden te bestuderen.

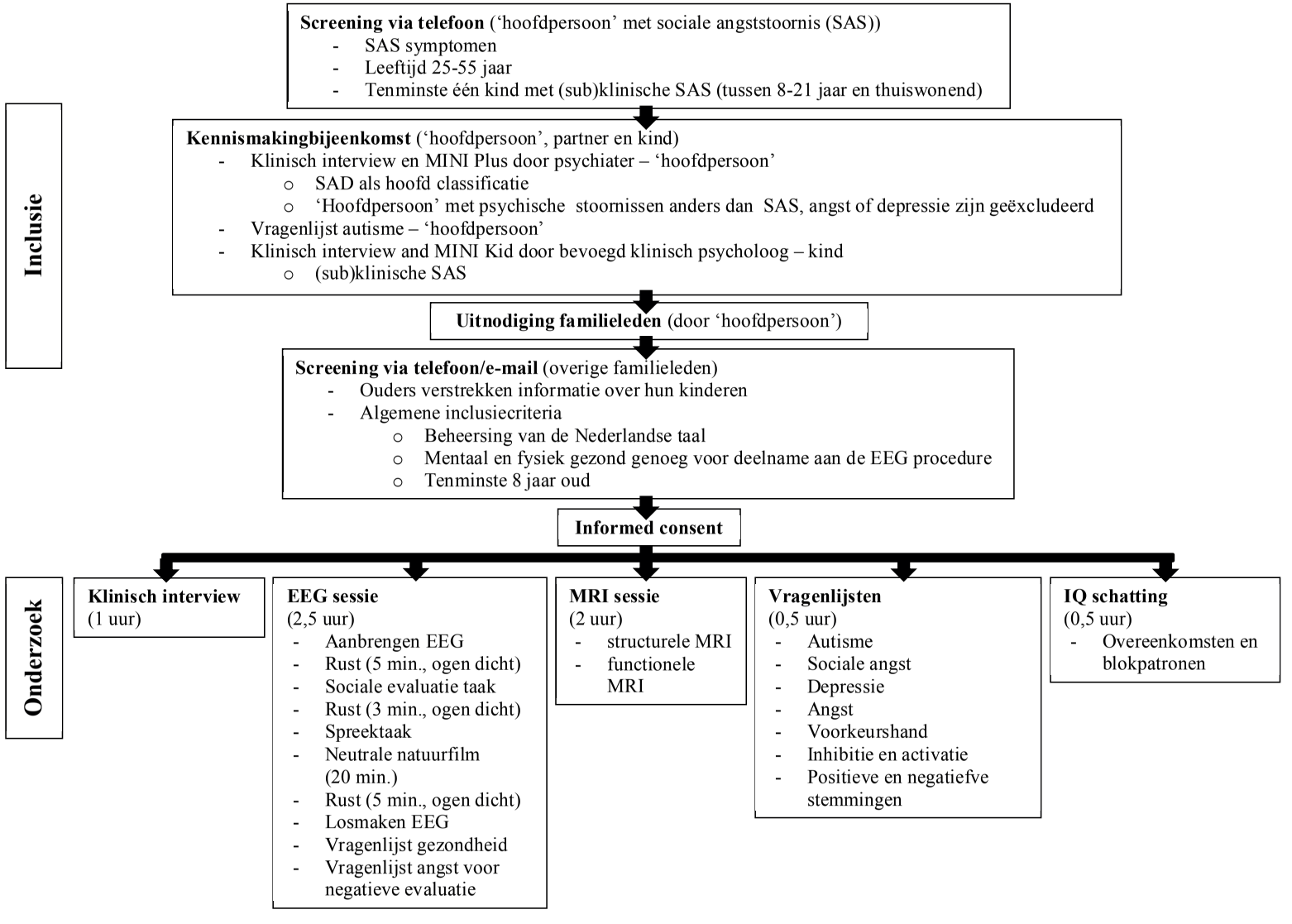
Leids familie-onderzoek

Het doel van dit unieke Leidse familie-onderzoek was om endofenotypes van sociale angststoornis te onderzoeken, door ons op het tweede (samengaan met de stoornis binnen families) en derde (erfelijkheid) criteria voor endofenotypes te richten. Het was hiervoor nodig om families van mensen met sociale angststoornis te onderzoeken, omdat we zo informatie konden halen uit de verschillende familiebanden binnen de families.

Aan dit onderzoek deden volwassen 'hoofdpersonen' met sociale angststoornis mee met hun partner en kinderen en hun broers/zussen met partner en kinderen (zie figuur 1). Ten minste één van de kinderen van deze 'hoofdpersonen' had ook last van sociale angst symptomen (dit hoefde niet per se écht de stoornis te zijn) en woonde thuis. Alle familieleden boven de 8 jaar werden uitgenodigd voor dit onderzoek.



Figuur 1. Voorbeeld van een fictieve familie uit het Leidse familie-onderzoek

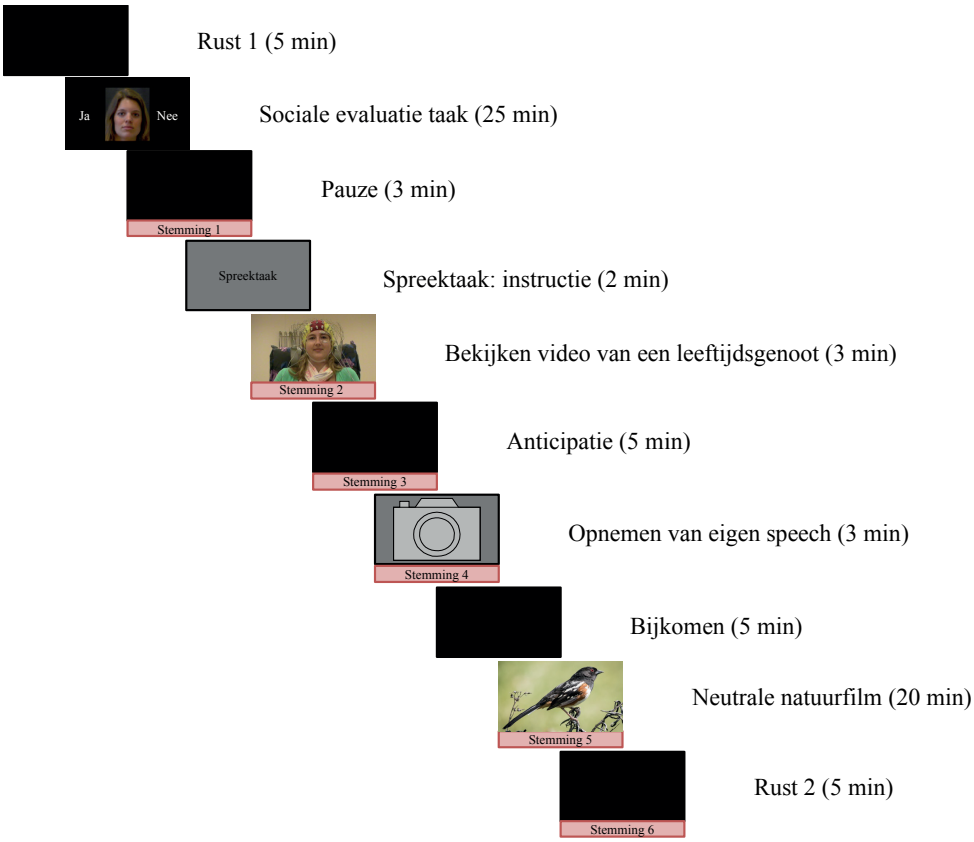

Inclusie
Screening via telefoon (‘hoofdpersoon’ met sociale angststoornis (SAS))
- SAS symptomen
- Leeftijd 25-55 jaar
- Tenminste één kind met (sub)klinische SAS (tussen 8-21 jaar en thuiswonend)
Kennismakingbijeenkomst (‘hoofdpersoon’, partner en kind)
- Klinisch interview en MINI Plus door psychiater – ‘hoofdpersoon’
  - SAD als hoofd classificatie
  - ‘Hoofdpersoon’ met psychische stoornissen anders dan SAS, angst of depressie zijn geëxcludeerd
- Vragenlijst autisme – ‘hoofdpersoon’
- Klinisch interview and MINI Kid door bevoegd klinisch psycholoog – kind
  - (sub)klinische SAS
Uitnodiging familieleden (door ‘hoofdpersoon’)
Screening via telefoon/e-mail (overige familieleden)
- Ouders verstrekken informatie over hun kinderen
- Algemene inclusiecriteria
  - Beheersing van de Nederlandse taal
  - Mentaal en fysiek gezond genoeg voor deelname aan de EEG procedure
  - Tenminste 8 jaar oud
Informed consent
Onderzoek
Klinisch interview
(1 uur)
EEG sessie
(2,5 uur)
- Aanbrengen EEG
- Rust (5 min., ogen dicht)
- Sociale evaluatie taak
- Rust (3 min., ogen dicht)
- Spreektaak
- Neutrale natuurfilm (20 min.)
- Rust (5 min., ogen dicht)
- Losmaken EEG
- Vragenlijst gezondheid
- Vragenlijst angst voor negatieve evaluatie
MRI sessie
(2 uur)
- structurele MRI
- functionele MRI
Vragenlijsten
(0,5 uur)
- Autisme
- Sociale angst
- Depressie
- Angst
- Voorkeurshand
- Inhibitie en activatie
- Positieve en negatiefve stemmingen
IQ schatting
(0,5 uur)
- Overeenkomsten en blokpatronen

Figuur 2. Overzicht van de verschillende onderdelen van het Leidse familie-onderzoek.

Alle familieleden werden gevraagd om deel te nemen aan de verschillende onderdelen van het onderzoek: klinisch interview, EEG sessie, MRI sessie, vragenlijsten en IQ maten (zie figuur 2). De volgorde van deze onderdelen verschilde tussen de deelnemers. Uiteindelijk hebben niet alle familieleden alles gedaan, omdat sommige mensen niet naar Leiden wilden komen of niet konden deelnemen aan EEG of MRI sessies door lichamelijke klachten. Mijn proefschrift richtte zich op de EEG sessie.

Tijdens de EEG sessie (zie figuur 3) maten we EEG en hartslag tijdens rust en twee verschillende taken. Deze taken hebben we gekozen omdat ze zich richten op een belangrijk aspect van sociale angststoornis: angst voor de beoordeling door anderen. In de sociale evaluatie taak ('social judgment paradigm') (Van der Molen et al., 2014) werden deelnemers gevraagd om eerst een foto van zichzelf op te sturen. We vertelden hen dat deze foto beoordeeld zou worden door een panel van leeftijdsgenoten (dat was niet het geval). Tijdens de EEG sessie kregen de deelnemers de foto's van deze panelleden te zien en vroegen we hen om aan te geven of ze verwachtten dat deze panelleden hun wel of niet aardig zouden vinden. Even later lieten we hen de 'beoordeling' van het panellid zien: deze persoon vond jou wel ('ja') of niet ('nee') aardig. Met deze taak konden we meten hoe vaak mensen aardig gevonden verwachten te worden en hoe zij reageerden op feedback (Van der Molen et al., 2014).

In de spreektaak ('social performance task') (Harrewijn et al., 2016) werden mensen gevraagd om een speech te geven. We lieten hen eerst een voorbeeldfilmpje zien van 'een andere deelnemer' die vertelde over haar goede en slechte eigenschappen (in werkelijkheid waren dit bekenden van mij). De deelnemers werden hierna gevraagd om de persoon op de video te beoordelen. Vervolgens kregen de deelnemers vijf minuten om na te denken over hun eigen goede en slechte eigenschappen. We vroegen hen toen om een filmpje van drie minuten te maken, die wij op een later moment aan een leeftijdsgenoot zouden laten zien. Die leeftijdsgenoot zou hun filmpje beoordelen op dezelfde manier als zij het voorbeeldfilmpje hadden beoordeeld. Dit was niet het geval, maar de deelnemers hadden echt het idee dat ze beoordeeld zouden worden. Ten slotte hadden de deelnemers vijf minuten om bij te komen (Harrewijn et al., 2016). We hebben EEG gemeten in de vijf minuten vlak voor en vlak na de speech, omdat mensen tijdens de speech teveel bewegen voor een goede EEG-meting. Met deze taak konden we meten hoe mensen reageerden in een stressvolle sociale situatie.



Figuur 3. Overzicht van de EEG sessie.

Het doel van dit proefschrift was om te kijken of verschillende maten tijdens rust, de sociale evaluatie taak en de spreektaak (gedrag, EEG en hartslag) endofenotypes van sociale angststoornis zijn. Dit is belangrijk om meer inzicht te krijgen in de mechanismes die een rol spelen in het ontwikkelen en in stand houden van sociale angststoornis. In het *tweede hoofdstuk* hebben we gekeken naar het eerste criterium voor endofenotypes (gerelateerd zijn aan stoornis), door een overzicht te geven van de meest onderzochte EEG maten van afwijkingen in de informatieverwerking. In het *derde hoofdstuk* beschrijven we onze nieuwe spreektaak en onderzochten we deze taak in hoog en laag sociaal angstige vrouwen. We hebben hierbij gekeken naar twee EEG maten die in eerdere studies zijn gebruikt: frontale alfa asymmetrie en delta-beta correlatie. De laatste drie hoofstukken focussen zich op de tweede (samengaan met de stoornis binnen families) en derde (erfelijkheid) criteria voor endofenotypes en beschrijven de bevindingen van het Leidse familie-onderzoek. In het *vierde hoofdstuk* beschrijven we of delta-beta correlatie tijdens de spreektaak gezien kan worden als

een endofenotype van sociale angststoornis. Het *vijfde hoofdstuk* focust op hartslagvariabiliteit tijdens rust en de spreektaak als een mogelijk endofenotype van sociale angstsstoornis. Het *zesde hoofdstuk* beschrijft of gedrags- en EEG-maten tijdens de sociale evaluatie taak gezien kunnen worden als endofenoypes van sociale angststoornis. Het *zevende hoofdstuk* is een discussie van de bevindingen uit dit proefschrift en bespreekt ideeën voor toekomstig onderzoek en de klinische implicaties van dit onderzoek.

Hoofdstuk 2 – EEG maten van afwijkingen in de informatieverwerking in sociale angststoornis: een review

Eerder onderzoek heeft laten zien dat mensen met sociale angststoornissen afwijkingen laten zien in de informatieverwerking, ze focussen zich bijvoorbeeld meer op negatieve sociale situaties dan andere mensen. De onderliggende mechanismes van deze afwijkingen zijn nog onduidelijk en EEG zou hier meer informatie over kunnen geven. Daarom hebben wij een overzicht gegeven van de meeste bestudeerde EEG maten tijdens rust, anticipatie, stimulus verwerking en bijkomen. Sociale angst was gerelateerd aan delta-beta correlatie tijdens het anticiperen op en bijkomen van stressvolle sociale situaties. Dit is waarschijnlijk een maat van minder goede communicatie tussen corticale en subcorticale breingebieden. Sociale angst was ook gerelateerd aan een verhoogde vroege reactie op gezichten en het maken van fouten. Deze verhoogde reactie op gezichten zou kunnen laten zien dan mensen met sociale angststoornis hun aandacht meteen richten op bedreigende stimuli. De verhoogde reactie op fouten zou kunnen laten zien dat mensen met sociale angststoornis meer aandacht op zichzelf richten (zich meer bewust zijn van zichzelf) of dat ze perfectionistisch zijn. Gezamenlijk zouden deze EEG maten deel kunnen uitmaken van een vicieuze cirkel die sociale angststoornis in stand houdt. Vervolgonderzoek zou moeten onderzoeken of deze maten (1) al op jonge leeftijd geïdentificeerd kunnen worden, (2) gebruikt kunnen worden om de ontwikkeling van sociale angststoornis te voorkomen, (3) een rol kunnen spelen in de ontwikkeling van sociale angst en (4) inzicht kunnen geven in genetische mechanismen van sociale angststoornis.

Hoofdstuk 3 – Mogelijke EEG maten van sociale angst: Een vergelijking van frontale alfa asymmetrie en delta-beta correlatie

Frontale alfa asymmetrie en delta-beta correlatie zijn twee EEG maten die beide zijn onderzocht in spreektaken in relatie tot sociale angst. Dit is de eerste studie waarin deze twee

maten tegelijkertijd onderzocht worden, andere onderzoeken richtten zich op slechts één van de twee maten. Het doel was om te kijken of frontale alfa asymmetrie en delta-beta correlatie tijdens rust, en tijdens de spreektaak verschilden tussen hoog en laag sociaal angstige vrouwen. Deelnemers deden onze spreektaak waarin ze eerst de video van een leeftijdsgenoot bekeken en beoordeelden en vervolgens zelf een speech voorbereidden. Zij dachten dat hun video bekeken en beoordeeld zou worden door een leeftijdsgenoot. We vonden dat hoog sociaal angstige deelnemers meer negatieve delta-beta correlatie lieten zien dan laag sociaal angstige deelnemers. Deze negatieve delta-beta correlatie reflecteert mogelijk verhoogde activatie in subcorticale breingebieden en verminderde activatie van corticale breingebieden. Hoog en laag sociaal angstigen verschilden niet in delta-beta correlatie tijdens rust. Dit zou kunnen betekenen dat een bepaald niveau van stress nodig is om EEG maten van sociale angst te vinden. Frontale alfa asymmetrie verschilde niet tussen hoog en laag sociaal angstigen, we beschrijven in het paper dat dit klopt met de tegengestelde bevindingen in de literatuur over frontale alfa asymmetrie. Kortom, onze resultaten suggereerden dat delta-beta correlatie een mogelijke EEG maat van sociale angst is.

Hoofdstuk 4 – Delta-beta correlatie als kandidaat endofenotype van sociale angst: een familie-onderzoek met twee generaties

In dit familie-onderzoek bestudeerden wij of delta-beta correlatie tijdens de spreektaak gezien kan worden als een mogelijk endofenotype van sociale angststoornis. Negen families met een 'hoofdpersoon' (gediagnosticeerd met sociale angststoornis), zijn/haar partner, kinderen en broers/zussen met hun partner en kinderen deden een spreektaak waarin ze een speech voor een camera moesten geven. EEG werd gemeten tijdens rust, anticipatie en bijkomen. Onze analyses richtten zich op twee criteria voor endofenotypes: samengaan met de stoornis binnen families en erfelijkheid. We vonden geen verschillen tussen mensen met en zonder sociale angststoornis, dus we richtten ons op (sub)klinische sociale angst. De groep mensen met (sub)klinische sociale angst bestond uit mensen met sociale angststoornis, maar ook mensen met veel klachten van sociale angst. Deelnemers met (sub)klinische sociale angst lieten meer negatieve delta-beta correlatie zien tijdens anticipatie dan deelnemers zonder (sub)klinische sociale angst. Delta-beta correlatie tijdens anticipatie was ook erfelijk. Delta-beta correlatie tijdens rust of bijkomen verschilde niet tussen deelnemers met en zonder (sub)klinische sociale angst. Delta-beta correlatie is mogelijk een endofenotype van sociale angst en reflecteerd waarschijnlijk de communicatie tussen corticale en subcorticale breingebieden.

Als het inderdaad een endofenotype blijkt te zijn, zou delta-beta correlatie gebruikt kunnen worden om meer te weten te komen over de genetische basis van sociale angststoornis. Daarnaast zou het een rol kunnen spelen in het verbeteren van behandeling en vroege detectie van mensen die verhoogd risico hebben op het ontwikkelen van sociale angststoornis.

Hoofdstuk 5 – Hartslagvariabiliteit als kandidaat endofenotype van sociale angst: een familie-onderzoek met twee generaties

Het doel van deze studie was om te onderzoeken of hartslagvariabiliteit tijdens rust en de spreektaak een kandidaat endofenotype is van sociale angststoornis. In totaal hadden we hartslagdata tijdens rust en de spreektaak van 121 familieleden (verdeeld over 9 families en totaal 18 patiënten met sociale angststoornis). Hartslagvariabiliteit is gemeten tijdens twee rustfases en tijdens het anticiperen, speechen en bijkomen. We testten twee criteria voor endofenotypes: samengaan met de stoornis binnen families en erfelijkheid. Hartslagvariabiliteit ging niet samen met sociale angststoornis binnen families. Een aantal hartslagvariabiliteit-maten waren wel erfelijk: RMSSD tijdens de eerste rustfase en bijkomen, 'high frequency power' tijdens alle fases van de taak, en LF/HF ratio tijdens anticiperen. Hartslagvariabiliteit tijdens rust en de spreektaak is een mogelijk endofenotype, maar niet van sociale angststoornis. Hartslagvariabiliteit zou een genetische kwetsbaarheid voor verschillende internaliserende stoornissen kunnen reflecteren en is waarschijnlijk gerelateerd aan minder flexibiliteit door minder inhibitie of een gegeneraliseerd gevoel van onveiligheid.

Hoofdstuk 6 – Gedrags- en EEG-reacties op sociale evaluatie: een familie-onderzoek met twee generaties over sociale angst

Het doel van deze studie was om endofenotypes (gemeten in gedrag of EEG) van sociale angst te vinden die gerelateerd zijn aan sociale evaluatie. Negen families deden de sociale evaluatietaak waarin ze dachten dat ze beoordeeld waren door leeftijdsgenoten. Voor elke leeftijdsgenoot gaven de deelnemers aan wat zij verwachtten van de beoordeling en zagen daarna of ze geaccepteerd of afgewezen waren door deze leeftijdsgenoot. Taakgedrag en EEG maten na de feedback (N1, FRN, P3 en theta power) werden getest als mogelijke endofenotypes. Hoeveel tijd mensen namen om aan te geven dat ze geaccepteerd dachten te worden was een kandidaat endofenotype van sociale angststoornis. Mensen met sociale angststoornis namen meer tijd omdat ze misschien onzekerder waren, meer aandacht richtten op zichzelf of extra oplettend waren tijdens de sociale evaluatie taak. N1 na verwachtte

afwijzing en P3 na acceptatie waren ook kandidaat endofenotypes van sociale angststoornis. De verhoogde N1 zou gerelateerd kunnen zijn aan verhoogde aandacht voor bedreigende sociale stimuli en de verhoogde P3 zou kunnen laten zien dat mensen met sociale angststoornis positieve feedback belangrijker vinden of minder hadden verwacht. Ten slotte, verhoogde FRN en theta power na onverwachte afwijzing vergeleken met de andere condities ging samen met sociale angststoornis binnen families, maar deze EEG maten waren niet erfelijk. De kandidaat endofenotypes spelen mogelijk een nieuwe en veelbelovende rol in toekomstig onderzoek naar genetische mechanismes, vroege detectie en/of preventie van sociale angststoornis.

Algemene discussie

In dit proefschrift heb ik mij gericht op drie criteria voor endofenotypes: is de maat gerelateerd aan de stoornis (*tweede hoofdstuk*), gaat de maat samen met de stoornis binnen families en is de maat erfelijkheid (*vierde, vijfde* en *zesde* hoofdstuk). Delta-beta correlatie, P1 en ERN voldeden aan het eerste criterium voor endofenotypes. Delta-beta correlatie, reactietijd, N1 en P3 voldeden aan de tweede en derde criteria voor endofenotypes. We hebben het vierde criterium 'familieleden die geen last van de stoornis hebben, moeten de maat meer laten zien dan mensen uit de normale populatie' en het vijfde criterium 'de maat moet onafhankelijk zijn van staat' niet getest. We moeten dus voorzichtig zijn met onze conclusie dat deze psychofysiologische maten endofenotypes van sociale angststoornis zijn.

Dit proefschrift geeft ook inzicht in welke maten niet geschikt zijn als endofenotypes van sociale angststoornis. Ten eerste, frontale alfa asymmetrie lijkt gerelateerd aan sociale angst of geïnhibeerd gedrag in kinderen (Fox et al., 2001; Henderson et al., 2001; Henderson et al., 2004), maar studies in volwassenen laten tegengestelde resultaten zien (zie *hoofdstuk twee*). Toen ik mijn resultaten besprak met andere onderzoekers, kwam ik erachter dat er meerdere mensen geen effect van sociale angst op alfa asymmetrie hadden gevonden, maar dit nooit hadden gepubliceerd. Het zou kunnen dat de inconsistente bevindingen in alfa asymmetrie veroorzaakt worden door depressie (Thibodeau et al., 2006). Ten tweede, eerder onderzoek heeft ook gemixte resultaten laten zien over hartslagvariabiliteit en sociale angst (zie *hoofdstuk vijf*). Hartslagvariabiliteit is gerelateerd aan verschillende internaliserende stoornissen, zoals verschillende angststoornissen (Chalmers et al., 2014; B. H. Friedman, 2007; Pittig et al., 2013) en depressie (Kemp et al., 2012; Kemp et al., 2010). Daarom hebben

wij in *hoofdstuk vijf* gesuggereerd dat hartslagvariabiliteit een genetische kwetsbaarheid reflecteert voor verschillende internaliserende stoornissen.

In de inleiding heb ik beschreven dat mensen met sociale angststoornis afwijkingen in de informatieverwerking laten zien. De kandidaat endofenotypes die we hebben gevonden in dit proefschrift, zouden deze afwijkingen kunnen reflecteren. Bijvoorbeeld, vroege EEG maten reflecteren mogelijk vroege aandacht voor bedreigende sociale stimuli en delta-beta correlatie laat mogelijk zien dat sociale situaties anders geïnterpreteerd worden. Het is natuurlijk nodig om deze relaties beter te onderzoeken in toekomstig onderzoek en om te onderzoeken hoe dit gebruikt kan worden in behandeling van sociale angststoornis.

Ideeën voor toekomstig onderzoek

Het onderzoeken van endofenotypes wordt gezien als een eerste stap in het ontrafelen van genen die betrokken zijn bij psychiatrische stoornissen, omdat endofenotypes waarschijnlijk worden beïnvloed door minder genen (Cannon & Keller, 2006; Glahn et al., 2007). Toekomstig onderzoek zou dus moeten onderzoeken welke genen gerelateerd zijn aan de psychofysiologische endofenotypes en of deze genen ook gerelateerd zijn aan sociale angststoornis. Hoewel, recente studies hebben dit gebruik van endofenotypes in twijfel getrokken, omdat endofenotypes nog steeds veroorzaakt worden door veel verschillende genen (Flint et al., 2014; Iacono et al., 2016). Desondanks kunnen endofenotypes meer inzicht geven in de biologische mechanismes die een rol spelen in sociale angststoornis (De Geus, 2010; Flint et al., 2014; Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013).

In dit proefschrift heb ik verschillende ideeën voor vervolgonderzoek gegeven. Ten eerste, het zou onderzocht moeten worden of de psychofysiologische maten elkaar beïnvloeden. Onderzoek heeft zich tot nu toe vooral gericht op verschillende afwijkingen in de informatieverwerking, maar het zou goed kunnen dat vroege EEG maten latere EEG maten beïnvloeden. Ten tweede, de link tussen psychofysiologische endofenotypes en gedrag zou verder onderzocht moeten worden. Het is bijvoorbeeld belangrijk om te weten hoe die vroege EEG reactie op gezichten het gedrag van mensen in contact met anderen beïnvloedt. Ten derde, het is belangrijk om te weten welke personen met een onderliggende genetische kwetsbaarheid de stoornis gaan ontwikkelen en welke personen niet. Ten vierde, het zou onderzocht moeten worden hoe de psychofysiologische endofenotypes zich in de loop van de tijd ontwikkelen.

Klinische implicaties

Het uiteindelijke doel van onderzoek naar psychofysiologische endofenotypes van sociale angststoornis is om meer inzicht te krijgen in de onderliggende mechanismes van deze stoornis om uiteindelijk vroege detectie en behandeling te verbeteren. Deze inzichten kunnen gebruikt worden om in een behandeling te focussen op de meest belangrijke onderliggende mechanismes van sociale angststoornis. Bijvoorbeeld, als de verhoogde vroege reactie op bedreigende sociale stimuli het belangrijkst blijkt te zijn, is het mogelijk om behandeling te richten op het verlichten van deze vroege reactie. Endofenotypes zouden mogelijk ook kunnen voorspellen welke behandeling het beste werkt voor welke patiënt. Het zou kunnen dat patiënten met een verhoogde vroege reactie een andere behandeling nodig hebben dan patiënten met problemen in tijdens anticiperen van sociale situaties. Ten slotte zouden endofenotypes een rol kunnen spelen in het vroeg detecteren van sociale angststoornis en het ontwikkelen van preventieve interventies. Als we al vroeg kunnen voorspellen welke kinderen een verhoogd risico hebben op het ontwikkelen van sociale angststoornis, zouden we vroeger kunnen ingrijpen en hoeft hun verlegenheid niet uit te groeien tot sociale angststoornis.

Sociaal angstige ouders waren erg gemotiveerd om deel te nemen aan ons familie-onderzoek omdat zij hun eigen angst herkenden in hun kinderen. Ze wilden niet dat hun kinderen dezelfde problemen zouden ontwikkelen. Ook de andere familieleden waren erg gemotiveerd om iets te doen voor hun familielid met sociale angststoornis. Veel proefpersonen kwamen bijvoorbeeld uit de andere kant van het land en reden drie uur (met hun kinderen op de achterbank) om deel te nemen aan ons onderzoek. Deze voorbeelden onderstrepen het belang van onderzoek naar sociale angststoornis en de noodzaak voor toekomstig onderzoek naar factoren die sociale angststoornis zouden kunnen verminderen.

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CV

Curriculum vitae

Anita Harrewijn was born in Delft (1990) and graduated from 'Hoeksch Lyceum' high school in Oud-Beijerland in 2008. She completed both her bachelor in psychology (2008-2011) and her research master in developmental psychology (2011-2013) at Leiden University. For her honours research bachelor project, she worked with dr. Dorien Enter on a study about the role of testosterone in social anxiety disorder. For her research master internship and thesis, she learned how to collect and analyze fMRI data in the Brain and Development lab of prof. Eveline Crone. Anita studied intentional inhibition in typically developing children and children who stutter with dr. Margot Schel. During her research master, she had the opportunity to visit the lab of prof. Patrick Haggard in London for two months to work on the development of a new social paradigm measuring intentional inhibition.

She completed her PhD research (2013-2017) under supervision of prof. Michiel Westenberg and dr. Melle van der Molen. The family study on social anxiety disorder was the most important part of her PhD, but she also conducted a study in high and low socially anxious students. She used several EEG and heart rate analysis techniques, and learned how to analyze complex family data. During her PhD, Anita visited the Child Emotion Laboratory of prof. Louis Schmidt to work on a review paper. Besides doing research, she also enjoyed teaching and obtained her certificate of basic teaching qualification (basis kwalificatie onderwijs).

In August 2017, Anita moved to the United Stated to work as a post-doctoral researcher with dr. Daniel Pine (National Institute of Mental Health) and prof. Nathan Fox (University of Maryland). She will work on a longitudinal study in children with a behaviorally inhibited temperament, and on several fMRI projects with children with anxiety disorders.

Publications

Harrewijn, A., Van der Molen, M.J.W., Van Vliet, I.M., Tissier, R.L.M., & Westenberg, P.M. (2018). Behavioral and EEG responses to social evaluation: A two-generation family study on social anxiety. *NeuroImage: Clinical, 17,* 549-562. doi: 10.1016/j.nicl.2017.11.010

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Manuscripts in preparation

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