Extremely shy & genetically close : investigating neurobiological endophenotypes of social anxiety disorder

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Part 1

The endophenotype concept in Social Anxiety Disorder

Chapter 1

Extremely Shy & Genetically Close –
an introduction

Imagine…

… you have to give an important presentation in front of an audience. You are the center of attention and all eyes are on you. How do you feel? Or: you are invited to a party where you don't know anyone. You enter the room and see that the other guests are already seated. What do you experience? Chances are you feel shy and uncomfortable in the beginning, tense maybe, but after a while these feelings will fade and you will have a good time. However, some people remain extremely nervous in social situations and even worry for days or weeks before, and after a social event. These people have an intense fear of being negatively evaluated, and are severely worried about doing something embarrassing in front of others. As a result, they try to avoid social situations as much as possible, and when they actually are in a social situation, they act like wallflowers and don't want to attract attention. This tendency could have a tremendous negative influence on their lives. These individuals suffer from a psychiatric condition: social anxiety disorder (SAD).

We know from previous research that this '*extreme shyness*' develops during childhood and early adolescence. In addition, the disorder often runs in families: being '*genetically close*' to a patient with SAD substantially increases the risk to develop the disorder. But which heritable characteristics make these children and adolescents more susceptible to developing SAD?

Investigating neurobiological endophenotypes of social anxiety disorder

In the novel 'Extremely Loud & Incredibly Close', the nine-year old Oskar Schell wanders through New York City in order to find the lock that belongs to a mysterious key that was owned by his father. His father lost his life in the attack on the World Trade Center on 9/11, and by his search Oskar tries to give meaning to his life (Safran Foer, 2005). This thesis also reflects a search, as the studies described in the present work investigate the behavioral and neurobiological profile of SAD, with a special focus on examining which characteristics are genetically linked to SAD. This is of importance, because these characteristics, the so-called endophenotypes, could provide more insight in the genetic vulnerability to develop SAD.

This chapter offers an introduction to SAD, as well as to the endophenotype concept. Furthermore, previous neuroimaging research on SAD with relevance to this thesis will be discussed. Finally, the studies included in this thesis will be briefly introduced.

Social anxiety disorder: phenotype, prevalence and development

As stated in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), patients suffering from SAD are characterized by a considerable fear or anxiety in social situations in which the possibility of evaluation by others is present (American Psychiatric Association, 2013). Examples of such situations are performance situations, like speaking, writing or eating in the presence of others, and situations involving social interactions, such as attending parties and meeting unfamiliar people (Furmark, 2002; Neal & Edelmann, 2003). In these social circumstances, patients fear that they will act in a way which will be negatively evaluated by other people, or that they will present themselves with anxiety symptoms like blushing or sweating. They are afraid that their performance in these situations will be humiliating or embarrassing, will lead to rejection by others or the offending of other people. As a result, patients with SAD avoid these social events or endure them with excessive fear or anxiety. Critically, the social situations must almost always elicit fear or anxiety and the fear, anxiety and avoidance should be persistent, lasting at least 6 months (American Psychiatric Association, 2013).

The lifetime prevalence of SAD is estimated between 6 and 13 percent (Bandelow & Michaelis, 2015; Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012; Ruscio et al., 2008; Stein et al., 2010), and recent data from the World Mental Health survey indicate that the disorder is prevalent across the globe (Stein et al., 2017). SAD often develops during late childhood and adolescence: studies demonstrated that the age of onset is around 10 years of age (Burstein et al., 2011; Ormel et al., 2014). However, the tendency to react to novel persons, experiences, and objects with wariness or avoidant behavior, is already observable in young babies; this characteristic propensity, which is called behavioral inhibition, reflects a stable and innate temperamental trait, and research has shown that behavioral inhibition in children is associated with an increased risk to develop SAD later in life (Clauss, Avery, & Blackford, 2015; Clauss & Blackford, 2012).

SAD is characterized by a persistent course, as shown in adolescents and young adults followed for ten years (Beesdo-Baum et al., 2012), and there is typically a long delay between the onset of the disorder and the first treatment contact (Iza et al., 2013). The effects of the disorder should not be underestimated: patients with SAD often experience problems at school and work, in activities with friends, and in their close relationships (Aderka et al., 2012; Dingemans, van Vliet, Couvée, & Westenberg, 2001; Hendriks et al., 2015; Russell & Topham, 2012). In addition, patients with SAD have an above-average risk of suffering from comorbid psychopathology, like mood disorders such as depression, addiction, and other anxiety disorders (Beesdo et al., 2007; Fehm, Pelissolo, Furmark, & Wittchen, 2005; Ohayon & Schatzberg, 2010), a tendency which is already present in adolescents suffering from SAD (Burstein et al., 2011). All together, these factors make SAD a very disabling condition, with

high costs for society (Acarturk et al., 2009; Dams et al., 2017; Hendriks et al., 2014; Stein & Kean, 2000; Stuhldreher et al., 2014; Wittchen, Fuetsch, Sonntag, Müller, & Liebowitz, 2000).

To avoid individual suffering and societal burden due to SAD as much as possible, effective preventive interventions are important (Craske & Zucker, 2001). To develop them, insight into the factors that make individuals vulnerable for developing SAD is needed. As argued by Beauchaine and colleagues, considering neurobiological processes is therefore essential (Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008). Indeed, previous research has revealed that various biological, together with psychological and social factors, interact in the development of SAD (Wong & Rapee, 2016). Family- and twin studies have, for example, indicated that SAD has a heritable component (Isomura et al., 2015; Mancini, Van Ameringen, Szatmari, Fugere, & Boyle, 1996; Middeldorp et al., 2005; Scaini, Belotti, & Ogliari, 2014; Stein, Chartier, Hazen, et al., 1998). Still, the genetic variations related to SAD are largely unknown and, for several reasons, not easy to find. First of all, the disorder differs from one patient to another (Hyett & McEvoy, 2018). In addition, it is assumed that multiple interacting genes play a role in the development of SAD (Domschke & Dannlowski, 2010; Meier & Deckert, 2019). As discussed in more detail in *Chapter* 2 of this thesis, this complicates the search for a link between social anxiety and genes (see also the review by Bearden, Jasinska, & Freimer (2009)). However, new approaches and new study designs can help to unravel this connection. An example is the application of the endophenotype concept, which is relatively new in psychiatry (Gottesman & Gould, 2003).

Endophenotype concept

Endophenotypes are measurable characteristics which are located between a phenotype, for instance social anxiety, and specific genetic variations. Examples of endophenotypes are changes in the structure and function of the brain, alterations in cognitive performance, and neurophysiological changes (Glahn, Knowles, et al., 2014). The following criteria are used to determine whether a characteristic is an endophenotype (Glahn, Thompson, & Blangero, 2007; Gottesman & Gould, 2003; Lenzenweger, 2013b; Puls & Gallinat, 2008): 1st the endophenotype should be *associated with the disorder of interest*; 2nd an endophenotype is supposed to be a *stable, state-independent trait, which is already present in a preclinical state*; 3rd an endophenotype should be *heritable*; 4th the endophenotype *co-segregates with the disorder within a family, with nonaffected family members showing altered levels of the endophenotype when compared to the general population*. As more extensively discussed in *Chapter 2*, it is assumed that endophenotypes are easier to detect than the underlying phenotype-related genotype. This way, endophenotypes could help in unraveling the genetic susceptibility to psychiatric disorders. Furthermore, endophenotypes have the potential to increase our un-

derstanding of the pathways leading to pathology (Flint, Timpson, & Munafò, 2014; Miller & Rockstroh, 2013). In addition, as endophenotypes are not necessarily uniquely related to one specific disorder, they could provide insight in the transdiagnostic characteristics of mental disorders (Beauchaine & Constantino, 2017; Miller & Rockstroh, 2013).

In the past decade, the endophenotype approach has been applied to psychiatric disorders like depression (Goldstein & Klein, 2014; Miskowiak et al., 2018), obsessive-compulsive disorder (Bey et al., 2018; de Vries et al., 2013; Vaghi et al., 2017), and schizophrenia (Blakey et al., 2018; Glahn, Williams, et al., 2014; Honea et al., 2008; McCarthy et al., 2018), revealing alterations in brain structure and function in patients as well as in their unaffected relatives. Thereby, these studies provide initial insight in the genetic vulnerability to these disorders, as they show that the changes are not just a manifestation of the disease-state (as the alterations were present in unaffected family members as well), and are likely heritable, because the characteristics were present in both patients and relatives (cf. (Ursu, 2017)). Research on endophenotypes of SAD is, however, still absent, although studies employing case-control designs have already provided valuable insight in the neurobiological changes related to SAD (*endophenotype criterion 1),* as will be summarized later in this chapter. Nevertheless, due to their focus on patients with SAD, these neuroimaging studies were not able to establish the *heritability* of these SAD-related brain characteristics (*endophenotype criterion 3)*, nor could they investigate the *co-segregation within families of probands (first element of endophenotype criterion 4)*. In other words, these studies revealed several *biomarkers* of SAD, being characteristics of brain function and brain structure related to a disorder, but not necessarily causally involved in the mechanistic pathway from genotype to phenotype (Lenzenweger, 2013a); however, whether these characteristics are candidate *endophenotypes* of SAD, and as such reflective of the genetic susceptibility to SAD, is still an open question. Given the heritable background of SAD, investigating whether these biomarkers qualify as endophenotypes could provide important additional knowledge to improve prevention and intervention for children and adolescents who are vulnerable to developing SAD due to their genetic make-up (Dick, 2018).

The Leiden Family Lab study on Social Anxiety Disorder (LFLSAD)

In a first effort to fill this gap in the scientific literature, we performed the Leiden Family Lab study on Social Anxiety Disorder (LFLSAD). As indicated by its name, the LFLSAD involves not only patients with SAD, but also their families, as family studies are particularly suitable to test two important endophenotype criteria and, as such, expand case-control studies (Glahn et al., 2018). First of all, a family design allows for examining whether a candidate endophenotype *co-segregates with the disorder within families* (first element of

endophenotype criterion 4). Furthermore, as multiple family members are investigated, the *heritability* of proposed endophenotypes can be determined (endophenotype criterion 3). In addition, family studies have enhanced statistical power to delineate associations between genotypes and phenotypes, and are cost-efficient (Glahn et al., 2018).

The LFLSAD aims to profile neurobiological endophenotypes of SAD, as measured with magnetic resonance imaging (MRI) and electroencephalography (EEG). The background and design of the study are outlined in *Chapter 3*. This thesis describes the results of several MRI studies which were part of the LFLSAD; the findings of the EEG session are reported in the thesis of Anita Harrewijn (Harrewijn, 2017). The MRI paradigms used within the LFLSAD are depicted in *Figure 1.1*. These particular paradigms were carefully chosen and developed based on the results of previous neuroimaging research on SAD biomarkers, as these studies provided evidence for the first endophenotype criterion of *association with the disorder*. In the following, I will briefly summarize these findings.

Neuroimaging research on SAD

In the last decades, neuroimaging research on biomarkers of SAD has expanded: while early imaging studies focused on key structures in the brain like the amygdala (Birbaumer et al., 1998) and subcortical areas (Potts, Davidson, Krishnan, & Doraiswamy, 1994; Schneider et al., 1999), more recent studies aimed to characterize SAD-related changes in both the structure and function of the whole brain.

To start, structural MRI studies comparing gray matter volumes between patients with SAD and healthy control participants demonstrated widespread alterations in the structure of the brain: changes were found in the frontal, temporal and parietal cortex, as well as in subcortical areas like the amygdala, thalamus and putamen (Brühl, Hänggi, et al., 2014; Irle, Barke, Lange, & Ruhleder, 2014; Meng et al., 2013; Talati, Pantazatos, Schneier, Weissman, & Hirsch, 2013). The studies described in *Chapter 4* and *Chapter 5* build on these insights and investigate whether structural brain characteristics are candidate endophenotypes of SAD (*Figure 1.1A*). We examined evidence for the endophenotype criterion of *co-segregation with the disorder within families* and established *heritability*.

In addition to these structural MRI studies, functional MRI (fMRI) studies yielded important insights in neurobiological characteristics related to SAD. FMRI studies use changes in the blood-oxygen-level dependent (BOLD) signal in order to obtain an estimation of neural activity. Most fMRI studies on SAD use stimuli which are anxiety-provoking for patients. Example of such stimuli are photographs of faces with negative or neutral expressions, stories describing social situations, or sentences involving personal feedback (Brühl, Delsignore, Komossa, & Weidt, 2014). Such stimuli elicited increased brain activation in patients with SAD in several brain areas, including the amygdala, insula and prefrontal

cortex. In addition, enhanced brain responsivity of the parietal cortex has been associated with SAD. The involvement of these areas in the pathophysiology of SAD was confirmed by the results of a meta-analysis on fMRI findings (Brühl, Delsignore, et al., 2014).

Notably, several fMRI studies provided evidence for correlations between these increases in brain activation and the level of social anxiety symptoms; for example, Frick and colleagues reported a positive correlation between amygdala reactivity to emotional faces and social anxiety severity (Frick, Howner, Fischer, Kristiansson, & Furmark, 2013). Such associations support the hypothesis that neurobiological brain alterations, as measured with MRI, underlie the thoughts and behavior associated with SAD and are valuable biomarkers of the disorder. This idea is also substantiated by studies investigating treatment effects in SAD. To illustrate, Phan and colleagues reported that the exaggerated amygdala response to fearful faces, which was present in patients with SAD before treatment, significantly reduced after a twelve-week treatment with the selective serotonin reuptake inhibitor sertraline (Phan et al., 2013). These findings indicate that fMRI studies yield important and relevant insights in the neurobiological brain alterations that are functionally related to SAD. *Chapters 8, 9 and 10* of this thesis extend these biomarker studies, by investigating whether these alterations in brain activity could be considered candidate SAD endophenotypes. We focused on two neurobiological processes which are highly relevant for SAD patients: the processing of neutral faces by the amygdala and the processing of social norm violations, a paradigm which primarily targeted the prefrontal cortex (*Figure 1.1C-D*). Again, we investigated the *co-segregation of brain activation with the disorder within families* and estimated *heritability*.

Another line of neuroimaging research on SAD investigates changes in the connections between brain regions. Such networks can be visualized using fMRI and diffusion tensor imaging (DTI). In this context, fMRI studies estimate functional connections by exploring correlations in brain activation patterns, based on the idea that connected regions show similar reactivity patterns (Damoiseaux et al., 2006). DTI scans map connections between areas by enabling reconstruction of white matter tracts (Chanraud, Zahr, Sullivan, & Pfefferbaum, 2010). Previous work, as summarized by Cremers and Roelofs (2016), suggests that SAD patients are characterized by changes in subcortical networks (Arnold Anteraper et al., 2014), in networks involved in social cognition and self-reflection (Heitmann et al., 2016; Liao, Chen, et al., 2010), as well as in the white matter tract that connects the amygdala and the prefrontal cortex (Baur et al., 2011; Baur, Hänggi, Langer, & Jäncke, 2013). Within the LFLSAD MRI paradigm, we acquired data on functional (resting-state) as well as on structural (DTI) connectivity (*Figure 1.1B, Figure 1.1E*). These data are presently analyzed, and not part of this thesis.

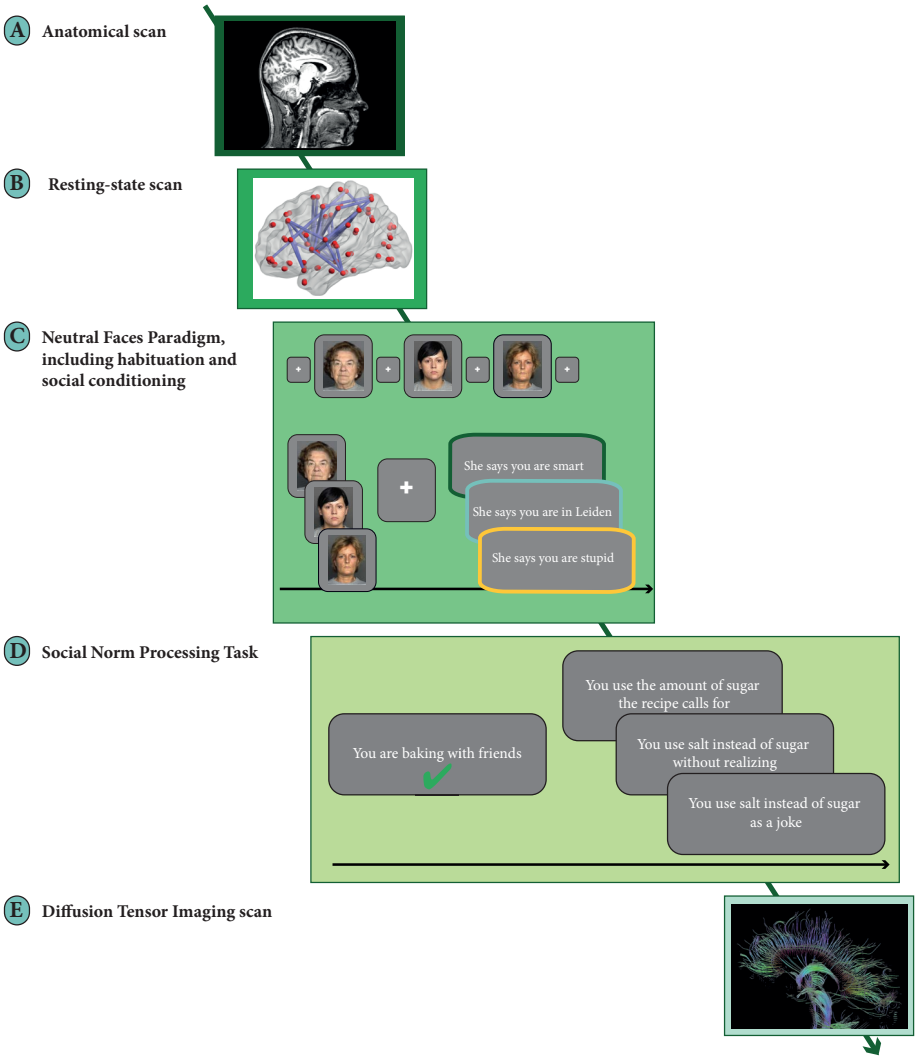

Figure 1.1 Magnetic resonance imaging (MRI) protocol of the Leiden Family Lab study on Social Anxiety Disorder.
A structural MRI scan, aimed to acquire a detailed anatomical scan of the brain (***Figure 1.1A***) and a resting-state scan (in which participants had their eyes closed; ***Figure 1.1B***) were followed by two functional (f)MRI paradigms: the Neutral Faces Paradigm (***Figure 1.1C***) and the Social Norm Processing Task - revised (***Figure 1.1D***). At the end of the scan protocol, diffusion tensor imaging (DTI) scans were acquired to visualize the structural connectivity of the brain (***Figure 1.1E***).

This thesis

The studies enclosed in this thesis aim to gain more insight in several neurobiological endophenotypes of SAD. In *Chapter 2*, I describe the endophenotype approach in detail, and consider existing evidence for neurobiological candidate endophenotypes of SAD, focusing on the function of the amygdala and medial prefrontal cortex, changes in brain structure, and on the connections between brain regions. We review to which extent previous studies provide support for these characteristics meeting the endophenotype criteria. As specific endophenotype studies on SAD are lacking, we used the findings of studies on SAD biomarkers, as well as results from work in healthy participants and animal studies, in order to create a summary of current evidence for neurobiological SAD endophenotypes. This overview substantiated the choice of MRI paradigms used in the LFLSAD.

Chapter 3 describes the design of LFLSAD. This study offers, due to its unique design involving patients with SAD and their family members of two generations, the opportunity to investigate which neurobiological characteristics *co-segregate with the disorder within families* (endophenotype criterion 4, element 1) and are *heritable* (endophenotype criterion 3).

The second part of this thesis addresses changes in brain structure related to SAD. In *Chapter 4*, I describe an international, multi-center mega-analysis on structural MRI scans of 174 patients with SAD and 213 healthy control participants. Within this sample, we investigated changes in gray matter between the groups by using voxel-based morphometry (VBM), establishing structural biomarkers of SAD. In *Chapter 5*, we build upon this work: we used data from the LFLSAD to examine whether gray matter characteristics like cortical thickness, cortical surface area and volumetric indices of subcortical brain structures are not just biomarkers, but also candidate endophenotypes of social anxiety (*Figure 1.1A)*.

In the third part of this thesis, I outline studies investigating neurobiological changes in brain function related to social anxiety, using two paradigms. The first paradigm, the revised Social Norm Processing Task (SNPT-R) concerns the processing of social norm violations and pays special attention to the intention underlying a social norm transgression, because intentional and unintentional social norm violations are contrasted (*Figure 1.1D*). This paradigm is highly relevant in the context of SAD, as it directly relates to the fear of SAD patients to behave in an embarrassing way in front of others. *Chapter 6* outlines the characteristics of the SNPT-R, which we developed in order to investigate the behavioral and neural correlates of processing social norm transgressions in children, adolescents as well as in adults. In *Chapter 7*, I summarize the results of a study on the relation between social anxiety and behavioral ratings on the SNPT-R in a sample from the general population. Subsequently, we used data from the LFLSAD to investigate whether behavioral and neural correlates of processing unintentional social norm violations are candidate endophenotypes of social anxiety. The results of this study are described in *Chapter 8*.

The second fMRI paradigm of the LFLSAD is described in *Chapter 9* and *Chapter 10*. In these chapters, I outline the Neutral Faces Paradigm (NFP), which was designed to investigate the association between social anxiety and brain activation related to the processing of neutral faces (*Figure 1.1C*). In the first part of the paradigm, neutral faces were repeatedly presented, and we used these data to examine whether the predicted decline in brain activation over time (the habituation response) is a candidate endophenotype of social anxiety. This study is summarized in *Chapter 9*. In the second phase of the NFP, the faces were paired with social-evaluative sentences which were either positive, negative or neutral. This enabled us to investigate brain activation in response to faces conditioned with a social-evaluative meaning. These findings are discussed in *Chapter 10*.

The results of the studies included in this thesis are summarized in *Chapter 11*. In a subsequent discussion, I reflect upon what the outcomes of the LFLSAD revealed about the genetic vulnerability to develop SAD. Furthermore, I provide suggestions for future research, and consider several important characteristics of the present work.