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

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Chapter 2

Neurobiological candidate endophenotypes of Social Anxiety Disorder

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Abstract

Social anxiety disorder (SAD) is a disabling psychiatric disorder with a complex pathogenesis. Studies indicated a genetic component in the development of SAD, but the search for genetic mechanisms underlying this vulnerability is complicated. A focus on endophenotypes instead of the disorder itself may provide a fruitful path forward.

Endophenotypes are measurable characteristics related to complex psychiatric disorders and reflective of genetically-based disease mechanisms, and could shed light on the ways by which genes contribute to the development of SAD. We review evidence for candidate MRI endophenotypes of SAD and discuss the extent to which they meet the criteria for an endophenotype, focusing on the amygdala, the medial prefrontal cortex, whole-brain functional connectivity and structural-anatomical changes. Strongest evidence is present for the primary endophenotype criterion of association between the candidate endophenotypes and SAD, while the other criteria, involving trait-stability, heritability and co-segregation of the endophenotype with the disorder within families, warrant further investigation. We highlight the potential of neuroimaging endophenotypes and stress the need for family studies into SAD endophenotypes.

Introduction

Social anxiety disorder (SAD) is a highly disabling disorder with an estimated life-time prevalence of 10 – 15 % (de Graaf, ten Have, van Gool, & van Dorsselaer, 2012; Hendriks et al., 2014; Stein & Kean, 2000; Wittchen et al., 2011). Patients with SAD have an extreme fear of being negatively evaluated in social situations and, as a result, avoid social events or endure them with excessive fear or anxiety (American Psychiatric Association, 2013). SAD usually has its onset during early adolescence (Beesdo-Baum et al., 2015; Haller, Cohen Kadosh, Scerif, & Lau, 2015) and is characterized by a rather chronic, unremitting course (Beesdo-Baum et al., 2012; Blanco et al., 2011; Scholten et al., 2016; Steinert, Hofmann, Leichsenring, & Kruse, 2013), a high association with comorbid psychopathology (Beesdo et al., 2007; Fehm et al., 2005; Kessler, Chiu, Demler, Merikangas, & Walters, 2005), and a reduced quality of life (Acarturk, de Graaf, van Straten, Have, & Cuijpers, 2008; Stein & Kean, 2000). In addition, the direct healthcare costs and indirect economic burdens of SAD, due to lost productivity and early retirement, are high (Acarturk et al., 2009; Fineberg et al., 2013; Gustavsson et al., 2011; Moitra, Beard, Weisberg, & Keller, 2011; Stuhldreher et al., 2014).

The high prevalence, chronic course, and substantial costs of SAD strongly highlight the need for effective preventive interventions and improved treatment options. Yet, our insight into the development of SAD is still rather limited, hindering the possibility to identify early markers for detection and prevention. Previous research points to a complex pathogenesis including genetic vulnerabilities, neurobiological alterations, environmental factors, and psychological mechanisms (Domschke, 2013; Hirshfeld-Becker, Micco, Simoes, & Henin, 2008; Kendler, Gardner, & Lichtenstein, 2008; Wong & Rapee, 2016). Because genetic and neurobiological markers are likely present very early in life, these are potential primary intervention targets. In the present narrative review, we will therefore focus on neurobiological mechanisms involved in the genetic vulnerability to social anxiety.

Genes and SAD

Family- and twin studies show that anxiety disorders are familial and moderately heritable, with heritability estimates for SAD around 50 % (Gottschalk & Domschke, 2016; Isomura et al., 2015; Middeldorp et al., 2005; Scaini et al., 2014; Smoller, 2015; Torvik et al., 2016). These findings are supported by animal studies, which reveal significant heritability of extreme early life anxiety in non-human primates (Fox, Oler, Shackman, et al., 2015; Fox & Kalin, 2014; Oler et al., 2010). Given this heritability, several studies searched for genes associated with SAD. An early genome-wide association (GWA) study, in a sample now considered to be relatively small (17 families, $n = 163$), reported involvement of regions on chromosomes 9, 14, 16 and 18 in SAD (Gelernter, Page, Stein, & Woods, 2004), a linkage-study suggested chromosome 13 as a potential susceptibility locus for 'specific or social phobia' (Fyer et al., 2012), while a recent meta-analysis of GWA-studies on anxiety disorders (the largest

to date, with > 18 000 participants) identified various novel susceptibility loci related to anxiety (Otowa et al., 2016). However, it should be noted that these loci are often very large and span up to hundreds of genes (Domschke, 2013). Recently, a more specific multilevel epigenetic study reported that decreased methylation of the oxytocin receptor (chromosome 3) was related to SAD and SAD-related traits (Ziegler et al., 2015), but research into epigenetic alterations is still in its infancy.

Although these findings for a genetic basis of SAD are promising, they have not yet been replicated. Furthermore, it needs to be investigated whether these genetic findings are specific for SAD, and whether they reflect risk factors for SAD or are rather compensatory changes in response to SAD (Ziegler et al., 2015). Thereby, the genes underlying the vulnerability to SAD are until now largely unknown.

The endophenotype approach

The search for SAD genes is complicated by the heterogeneity of the disorder and the fact that the diagnosis is based on clinical assessments and not on biologically-based measurements (Bearden, Reus, & Freimer, 2004; Glahn et al., 2007; Gottesman & Gould, 2003). In addition, SAD is a polygenic disorder: multiple genetic variants, each with a relatively small effect, interact and lead to disease vulnerability (Binder, 2012; Domschke & Dannlowski, 2010; Fox & Kalin, 2014). These genetic variants are in turn influenced by environmental factors (Gottschalk & Domschke, 2016), further complicating the search for the genetic basis of SAD.

To facilitate the investigation of genetic factors in psychiatric disorders, the endophenotype approach has increasingly received attention (Glahn, Knowles, et al., 2014). Endophenotypes are measurable characteristics that form a causal link between genes and diseases, and are manifestations of underlying disease liability (Lenzenweger, 2013b) (*Figure 2.1*). Criteria used to define endophenotypes are the following (Glahn et al., 2007; Gottesman & Gould, 2003; Lenzenweger, 2013b; Puls & Gallinat, 2008): 1st *association with the disorder*; 2nd being a *stable, state-independent trait, which is already present in a preclinical state*; 3rd being *heritable*; 4th *co-segregation with the disorder within a family, with nonaffected family members showing altered levels of the endophenotype when compared to the general population*. In addition, an endophenotype is ideally more strongly associated with the disorder of interest in comparison to other psychiatric conditions (Lenzenweger, 2013a), but it is also possible that a certain endophenotype affects more than one disorder (Cannon & Keller, 2006) (see the *Discussion* of this Chapter for a more in-depth debate).

Originally, the usefulness of endophenotypes was supposed to lie in discovering the genes predisposing for complex disorders, based on the assumption that endophenotypes have a simpler genetic architecture than the disorders themselves (Glahn et al., 2007; Gottesman & Gould, 2003). This idea was, however, challenged by the results of a meta-analytic review (Flint & Munafò, 2007) which compared the effect sizes of genetic loci contributing

to psychiatric disorders (phenotypes) and loci contributing to endophenotypes. Results showed comparable effect sizes, so the assumption that endophenotypes have a simpler genetic architecture than phenotypes was not supported (Flint & Munafò, 2007). Recently, the findings of this meta-analysis were empirically confirmed by comparing GWA studies investigating the genetic effects related to endophenotypes of schizophrenia (for example, variation in brain structure and measures of cognitive performance) to studies aimed to identify risk genes for the disorder itself (Flint et al., 2014). Again, similar effect sizes were found. So, it is not necessarily true that the genetic architecture of endophenotypes is less complex than that of the disorders themselves (Flint et al., 2014; Glahn, Knowles, et al., 2014; Puls & Gallinat, 2008).

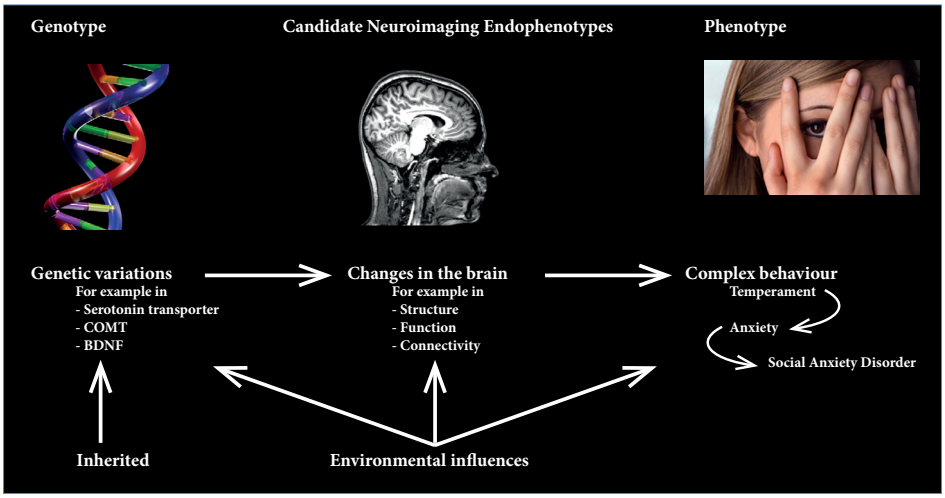

Figure 2.1 The relationship between genetic variation, endophenotype and phenotype.
Inspired by Kendler & Neale (2010). Illustration DNA: Wikimedia Commons, National Human Genome Research Institute, ID 85329. Photograph: *www.smartgirlsgroup.com*

This does not mean, however, that endophenotypes are of limited value. Their usefulness lies in understanding disease mechanisms: based on the assumption that complex disorders could be divided into simpler and more biologically coherent units (endophenotypes), endophenotypes could provide insight into the pathways leading to pathology and could help in discerning the origins of mental disorders (Flint et al., 2014; Miller & Rockstroh, 2013). Furthermore, endophenotypes could support a transdiagnostic perspective on mental disorders, given the fact that endophenotypes could cross traditional diagnostic boundaries (Miller & Rockstroh, 2013). Here, the endophenotype approach fits within the NIMH Research Domain Criteria (RDoC) initiative, a research framework in which not the clinical diagnoses are starting point for investigation, but core features of psychopathology, falling within five research domains (Sanislow et al., 2010). The RDoC initiative explicitly acknowledges that these core features could be present in multiple psychiatric disorders

and promotes the integration of data from several levels, from genes to neural systems to behavior. Goal of this approach is to classify disorders based on 'a deeper understanding of the biological and psychosocial basis' of psychiatric diseases (Insel, 2014). Endophenotypes could be used in this approach, because they provide a bridge between genetic variations at the one hand and psychiatric disorders at the other.

In addition, endophenotypes could aid in the development of improved animal models for psychopathology (Gould & Gottesman, 2006), and, based on the fact that endophenotypes are present prior to disease onset, endophenotypes can be used to identify individuals at risk (Puls & Gallinat, 2008). This is of uttermost importance, given the fact that early detection of psychopathology and subsequent use of preventive interventions can improve long-term prognosis, reduce the substantial burden and cost of SAD, and lower the risk of developing co-morbid psychopathology (Beauchaine et al., 2008). Furthermore, endophenotypes could provide clues for improvement of treatments for psychiatric disorders and guide in the selection of appropriate pharmacological interventions (Garner, Möhler, Stein, Mueggler, & Baldwin, 2009).

The endophenotype approach has been used successfully to investigate the genetic basis of several psychiatric disorders including depression (Goldstein & Klein, 2014; Hasler & Northoff, 2011), schizophrenia (Glahn, Williams, et al., 2014; Sutcliffe, Harneit, Tost, & Meyer-Lindenberg, 2016), bipolar disorder (Fears et al., 2015) and obsessive-compulsive disorder (Menzies et al., 2007), but research on endophenotypes for SAD is still in its infancy.

Review objectives

In this narrative review of empirical research from various sources, we explore potential candidate endophenotypes of social anxiety. We will focus on neurobiological measurements from magnetic resonance imaging (MRI) – a safe, non-invasive, widely applied and relatively accessible method used to investigate the structure and function of the human brain –, based on the assumption that changes in brain structure and function underlie thoughts and behavior associated with anxiety and anxiety disorders. In addition, we will refer to results from positron emission tomography (PET) studies, a method used for in vivo molecular imaging (Vaquero & Kinahan, 2015). Candidate endophenotypes were selected based on recent neuroimaging work on SAD (Brühl, Delsignore, et al., 2014) and include: function and connectivity of the amygdala, function of the medial prefrontal cortex, whole-brain network function and brain structure. We will qualitatively assess the potential of each candidate endophenotype using the four criteria listed in *Table 2.1* and illustrated in *Figure 2.2*.

The *association with disorder* (criterion 1) will be evaluated by discussing neuroimaging research that compares SAD patients with healthy participants. Given the fact that the candidate endophenotypes were selected based on studies on SAD, we expect relatively strong evidence for this criterion. We will briefly outline the results summarized by Brühl,

Table 2.1 Criteria for an endophenotype (EP).

1	EP is associated with the disorder: present in patients at a significantly different level than in general population
2	EP is a trait characteristic and already present in a preclinical state, reflecting the genetically-based vulnerability to the disorder
3	EP is heritable
4	EP co-segregates with the illness within a family, and nonaffected family members show altered EP levels when compared to the general population

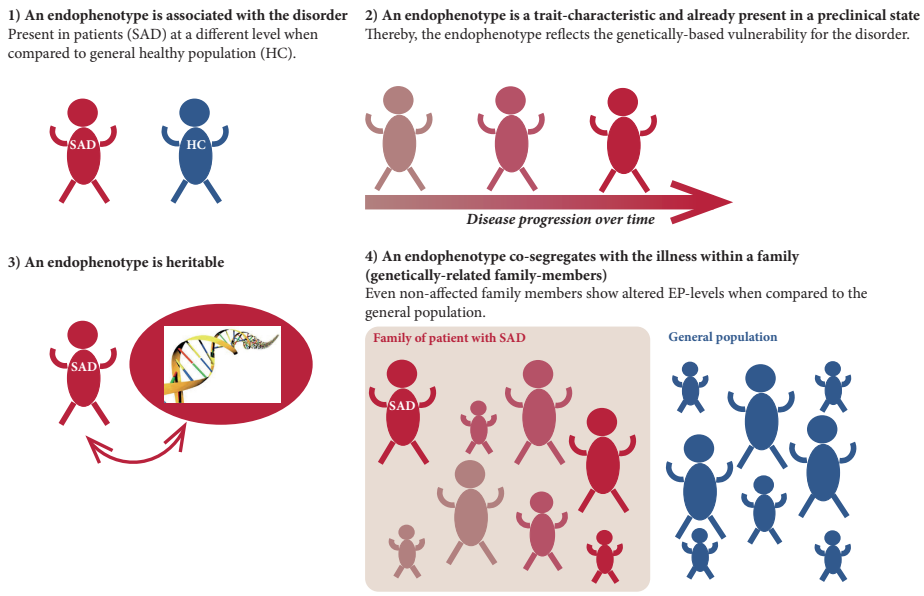

Figure 2.2 Illustration of the four criteria for an endophenotype.

Delsignore, et al. (2014) and extend these findings by summarizing findings of recently published work.

The *trait-stability* of an endophenotype (criterion 2) is ideally examined using longitudinal studies on individuals with SAD, while the *heritability* of an endophenotype (criterion 3) could be estimated from twin-, adoption or family studies. The fourth criterion, the *co-segregation of the endophenotype with illness within families*, is best investigated using studies in families genetically enriched for SAD. However, to the best of our knowledge, there are no longitudinal neuroimaging studies assessing the trait-stability of candidate endophenotypes of SAD, and family studies involving neuroimaging measurements of patients with SAD as well as of their family members, are lacking as well. Direct support for criteria 2, 3 and 4 is therefore mainly absent.

Therefore, we will explore available findings related to these criteria by using a broader perspective, in order to summarize indirect evidence for the candidate endophenotypes. Whether the candidate endophenotypes are *trait characteristics* (criterion 2) will be discussed based on studies investigating the relationship between the candidate endophenotype and several trait characteristics which are assumed to be more or less stable over time. In this light, it is especially useful to look at neuroimaging studies on behavioral inhibition or inhibited temperament. Inhibited temperament is the relatively stable tendency to withdraw from new and unfamiliar objects, situations and people, is already measurable in toddlers, and extreme behavioral inhibition is considered to be a risk factor for SAD (Clauss et al., 2015; Clauss & Blackford, 2012; Essex, Klein, Slattery, Goldsmith, & Kalin, 2010; Henderson, Pine, & Fox, 2015; Rapee, 2014). In addition, we will explore the results of neuroimaging studies on extraversion, a heritable personality trait that is negatively correlated with social anxiety (Bienvenu, Hettema, Neale, Prescott, & Kendler, 2007; Cremers & Roelofs, 2016; Kotov, Gamez, Schmidt, & Watson, 2010; Naragon-Gainey & Watson, 2011). Furthermore, animal studies and longitudinal studies on healthy participants could give insight into the trait-like characteristics and stability of candidate endophenotypes. It is, however, important to realize that measurements of brain activation as assessed by functional (f)MRI are inherently state-dependent, as they reveal the reactivity of brain regions in response to a task or during a certain period of rest. However, we assume that these reactivity patterns, as measured in the MRI scanner, are also reflective of a stable pattern of brain responses in a participant's daily life.

Evidence for *heritability* (criterion 3) will be investigated by describing studies on genetic influences for the candidate endophenotype, for example by summarizing results from studies describing heritability estimates of MRI measurements, and by discussing findings on genetic polymorphisms influencing brain function and structure. In addition, evidence on the *co-segration of the candidate endophenotype with the illness within families* (criterion 4) will be summarized when available.

Amygdala: function and functional connectivity

Neuroimaging research on SAD has generally focused on the amygdala, a key part of a broader circuit, known as the extended amygdala, which includes amygdala sub-nuclei like the central nucleus and basolateral complex, the bed nucleus of the stria terminalis (BNST), and the shell of the nucleus accumbens (Heimer & Van Hoesen, 2006; Janak & Tye, 2015). Although these subregions are functionally heterogeneous (LeDoux, 2007), the majority of past scientific imaging work in humans is based on the whole amygdala region, as imaging of the amygdala is difficult due to magnetic susceptibility differences and most imaging sequences do not have adequete spatial resolution to pinpoint amygdala subnuclei (Robinson,

Windischberger, Rauscher, & Moser, 2004) (but see for recent work with improved imaging parameters (Hrybouski et al., 2016)).

The amygdala is well known for its role in detecting cues that are predictive of potential threats (Fox, Oler, Tromp, Fudge, & Kalin, 2015; Hariri & Whalen, 2011) and individual differences in amygdala functioning are related to the etiology of anxiety (Shackman et al., 2016).

Criterion 1

Heightened amygdala reactivity in response to novel faces is consistently associated with SAD (Birbaumer et al., 1998; Blair, Geraci, Korelitz, et al., 2011; Evans et al., 2008; Fonzo et al., 2015; Hahn et al., 2011; Klumpp, Angstadt, Nathan, & Phan, 2010; Sladky et al., 2012; Stein, Goldin, Sareen, Zorrilla, & Brown, 2002; Straube, Kolassa, Glauer, Mentzel, & Miltner, 2004; Yoon, Fitzgerald, Angstadt, McCarron, & Phan, 2007). This is indicative of an exaggeration of the healthy response to novel and salient stimuli. In addition, increased amygdala reactivity in SAD patients has been reported in studies using SAD-specific symptom-provoking paradigms, like anticipation of giving a speech (Boehme, Ritter, et al., 2014; Lorberbaum et al., 2004; Tillfors, Furmark, Marteinsdottir, & Fredrikson, 2002), giving a speech (Tillfors et al., 2001), reading sentences containing self-referential criticism (Månsson et al., 2016) or receiving peer feedback (Guyer et al., 2008), as well as during unspecific tasks like anticipating (Brühl et al., 2011) or perceiving negative emotional images (Shah, Klumpp, Angstadt, Nathan, & Phan, 2009). Two recent meta-analyses confirmed that increased amygdala reactivity is observed in SAD (Brühl, Delsignore, et al., 2014; Gentili et al., 2016). In addition, Blair and colleagues reported that both adult as well as adolescent SAD patients demonstate amygdala hyperreactivity, supporting the assumption that perturbations in amygdala activation are present over the course of the disorder (Blair, Geraci, Korelitz, et al., 2011).

It is important to note that the amygdala does not function in isolation but is connected with other brain areas (Fox, Oler, Tromp, et al., 2015; Kim et al., 2011; LeDoux, 2007). Amygdala connectivity has been interrogated in SAD patients, using both resting-state methods to assess intrinsic connectivity and various tasks to assess functional task-based connectivity. Several studies showed differences in amygdala connectivity with a variety of brain regions. Resting-state studies demonstrated lower connectivity between the amygdala and the inferior temporal gyrus (Liao, Qiu, et al., 2010), the orbitofrontal cortex (Hahn et al., 2011) and the anterior cingulate cortex (Prater, Hosanagar, Klumpp, Angstadt, & Phan, 2013). Task-based studies using emotional faces show a functional disruption in the negative feedback loop between the amygdala and OFC (Sladky et al., 2015), increased connectivity between amygdala and the fusiform gyrus (Frick et al., 2013a) and increased positive coupling between the amygdala and the dorsal medial prefrontal cortex (Robinson et al., 2014). Furthermore, Cremers and colleagues found a transient decreased negative

functional connectivity between the amygdala and cortical regions involved in emotion regulation during anticipation of giving a public speech (Cremers et al., 2014). The divergent findings of these connectivity studies could be partly explained by the different conditions (rest or task), but in order to use amygdala connectivity as a reliable endophenotype of SAD, more research in larger samples is needed to establish which connectivity changes are consistently associated with SAD.

Criterion 2

There are several lines of evidence suggesting that amygdala functioning is a trait characteristic. First, research on healthy participants shows that inter-subject variability in temperamental traits, which are considered to be more or less stable over time, relates to differences in amygdala function and connectivity. For example, amygdala hyperreactivity was present in young adults with anxiety-related temperamental traits (Stein, Simmons, Feinstein, & Paulus, 2007). The most consistent relation is reported between inhibited temperament and hyperactive amygdala response to stimuli (for a review and meta-analysis see Clauss et al., 2015). To illustrate, adolescents with an inhibited temperament had an exaggerated amygdala response to emotional faces (Pérez-Edgar et al., 2007), and adults who had been characterized as inhibited at the age of two show elevated amygdala reactivity in response to novel faces (Schwartz, Wright, Shin, Kagan, & Rauch, 2003). Furthermore, behavioral inhibition during childhood predicts negative amygdala-frontal connectivity during an attention-bias task involving angry faces in young adulthood (Hardee et al., 2013). Another study demonstrated changes (both increases as well as decreases) in resting-state functional connectivity between subnuclei of the amygdala and the prefrontal cortex, striatum, anterior insula, and cerebellum in young adults with a history of behavioral inhibition (Roy et al., 2014). In addition, a high degree of social inhibition was associated with reduced resting-state connectivity between the superficial amygdala and the rostral cingulate cortex, and between the centromedial amygdala and the dorsal anterior cingulate cortex (Blackford et al., 2014). Furthermore, trait anxiety (and not state anxiety) predicted lower intrinsic functional connectivity between the amygdala and the entire cerebral cortex (He, Xu, Zhang, & Zuo, 2015). Findings on the relation between trait extraversion and amygdala activation are mixed, as both positive (Canli et al., 2002) as well as negative associations (Hooker, Verosky, Miyakawa, Knight, & D'Esposito, 2008) between extraversion and amygdala reactivity are reported (for a comprehensive review, see Kennis, Rademaker, & Geuze (2013). Studies on the functional connectivity of the amygdala revealed increased functional connectivity between the amygdala and brain regions involved in reward processing (Aghajani et al., 2014; Rohr et al., 2015) related to extraversion.

The relation between amygdala function and temperamental traits has been further explored in a specific line of research focusing on amygdala habituation. Studies in healthy participants have indicated that the amygdala response to facial stimuli declines when the

stimuli are presented repeatedly without meaningful consequences. This process, called habituation, is one of the most basic forms of social learning (Blackford, Allen, Cowan, & Avery, 2013; Zald, 2003). Amygdala habituation can be reliably assessed using fMRI (Plichta et al., 2014). Importantly, habituation is an adaptive process, because it enables individuals to focus their attention on novel stimuli with potential meaningful information. A failure to habituate may reflect inefficient processing of novel information. More specifically, a failure to habituate to social stimuli results in a sustained and heightened amygdala response, which may contribute to feelings of anxiety and uncertainty in unfamiliar social situations. Therefore, several research groups have investigated the relationship between amygdala habituation and inhibited temperament (Beaton et al., 2008; Blackford et al., 2013; Blackford, Avery, Cowan, Shelton, & Zald, 2011; Schwartz et al., 2012). These studies demonstrated an increased response to familiarized faces and a failure of the amygdala to habituate in response to repeatedly presented faces, in participants with an inhibited temperament (Blackford et al., 2013, 2011; Schwartz et al., 2012) and shy adults (Beaton et al., 2008). These results strengthen the idea that a more intense and prolonged amygdala response to familiar faces represents a neural substrate underlying the timid and anxious behavior of inhibited people, and, because of the relationship between inhibited temperament and SAD (Clauss & Blackford, 2012), provide evidence for impaired amygdala habituation as an endophenotype for SAD. Additional support comes from a study showing decreased habituation (Schneider et al., 1999) and an increased amygdala response during habituation in SAD patients (Veit et al., 2002), but it should be noted that two other studies did not provide evidence for failed habituation in SAD (Campbell et al., 2007; Sladky et al., 2012). However, these latter studies did not use a passive viewing design which could explain why these studies did neither provide evidence for failed habituation in SAD nor for 'normal' habituation in healthy participants (Campbell et al., 2007; Sladky et al., 2012).

The findings on the relationship between temperamental traits and amygdala functioning in healthy participants are paralleled by results from animal research, demonstrating a trait-like pattern of amygdala activation independent of context: young rhesus monkeys with anxious temperament (AT) show increased amygdala reactivity both in a stressful as well as in a safe context, suggesting that amygdala hyperreactivity is a stable characteristic of AT (Fox, Shelton, Oakes, Davidson, & Kalin, 2008). In addition, amygdala hyperreactivity is associated with multiple dimensions of AT (Shackman et al., 2013), and reduced functional connectivity between the amygdala and prefrontal cortex was reported in anxious monkeys as well as in anxious children (Birn et al., 2014). These findings confirm the evidence from human studies that amygdala hyperreactivity and reduced functional connectivity are trait characteristics of anxious temperament.

There is also evidence for additional state-influences on amygdala reactivity, both in healthy participants as well as in SAD patients. This does, however, not conflict with the trait-stability of endophenotypes: it is acknowledged that a specific challenge (for example,

participating in an experiment in the case of SAD) can reveal an endophenotype (Gould & Gottesman, 2006; Lenzenweger, 2013a). The level of social anxiety in healthy participants influenced amygdala reactivity during social conditioning (Pejic, Hermann, Vaitl, & Stark, 2013), while Brühl and colleagues reported that both the level of trait anxiety as well as the state-dependent level of social anxiety symptoms correlated with reactivity of the amygdala in SAD patients (Brühl et al., 2011). In addition, a significant but weak association between the intensity of social anxiety symptoms and left amygdala reactivity in SAD patients has been demonstrated (Shah et al., 2009).

Findings from studies on the effect of interventions on amygdala reactivity in SAD are mixed. Amygdala activation decreased when patients applied emotion regulation (Brühl, Herwig, Delsignore, Jäncke, & Rufer, 2013), as a result of internet-delivered cognitive behavioral therapy (CBT) (Månsson et al., 2013, 2016) and due to the use of selective serotonin reuptake inhibitors (Faria et al., 2012; Phan et al., 2013) or treatment with oxytocin (Labuschagne et al., 2010). Furthermore, oxytocin modulated functional connectivity of the amygdala (Gorka et al., 2015), and symptom improvement due to treatment with either citalopram or CBT was shown to reduce regional blood flow in the bilateral amygdala (Furmark et al., 2002). However, amygdala responsiveness was not related to treatment outcome in another study using CBT (Klumpp, Fitzgerald, & Phan, 2013).

Criterion 3

As far as we are aware of, there are no studies that directly investigated the heritability of amygdala functioning in healthy participants or patients with SAD, for example using twin- or family studies. However, three lines of evidence point towards genetic influences on amygdala functioning.

To start, various studies in healthy participants have indicated that genes involved in monoaminergic neurotransmission, for example the serotonin transporter gene variation (*5-HTTLPR*), the catechol-o-methyl transferase (*COMT*) gene and the monoamine oxidase A (*MAO-A*) gene influence amygdala functioning (Domschke et al., 2012; Hariri et al., 2002; Kempton et al., 2009; Lonsdorf et al., 2011; Rao et al., 2007). These effects have been confirmed by research on multiple species (Akimova, Lanzenberger, & Kasper, 2009; Caspi, Hariri, Holmes, Uher, & Moffitt, 2010) and by several meta-analyses (Munafò, Brown, & Hariri, 2008; Murphy et al., 2013). However, it should be noted that a recent study was unable to replicate the effect of *5-HTTLPR* variation on amygdala reactivity (Bastiaansen et al., 2014), which could be explained by the rather small contribution of this gene-variant to amygdala reactivity and the fact that the effect may be overestimated due to publication biases (Bastiaansen, de Vries, & Munafò, 2015; Murphy et al., 2013).

In addition, several studies investigated the effect of *5-HTT* genetic variation in SAD patients, confirming the relationship between carrying the short allele of this gene and increased amygdala reactivity (Battaglia et al., 2012; Furmark et al., 2004, 2009) and showing

a link between serotonin-related genotype, amygdala response and the effect of placebo-induced relief of SAD (Furmark et al., 2008). Furthermore, resting-state PET-studies on SAD patients reported reduced binding of the serotonin-1A receptor (Lanzenberger et al., 2007) and increased serotonin synthesis and transporter availability in the amygdala (Frick et al., 2015), a finding recently replicated in an independent sample in which a functional relation between serotonin formation and the tryptophan hydroxylase-2 (*TPH2 G-703T*) polymorphism was reported (Furmark et al., 2016). However, a linkage study in 122 first-degree family members of SAD patients did not yield evidence for a link between SAD and the *5-HTT* gene (Stein, Chartier, Kozak, King, & Kennedy, 1998).

A third line of evidence comes from research on the relation between amygdala activation, genetic variation and AT. Smoller and colleagues demonstrated that variations in the gene encoding the regulator of G protein signaling 2 (*RGS2*), a quantitative trait locus previously linked to anxious behavior in mice, were associated with the level of introversion (a personality trait related to SAD) as well as with the level of amygdala responsiveness during emotion processing, accounting for 15 % of the variance in amygdala activation in humans (Smoller et al., 2008). Converging evidence for a genetic influence on amygdala functioning in relation with AT comes from research on rhesus monkeys (Fox & Kalin, 2014). Studies showed altered expression of genes involved in amygdalar neuroplasticity in anxious young monkeys (Fox et al., 2012), demonstrated AT-related changes in neuropeptide Y gene receptor in the amygdala (Roseboom et al., 2014) and identified several genes with AT-associated methylation changes in the central nucleus of the amygdala (Alisch et al., 2014). In addition, significant heritability of AT-related glucose metabolism in the extended amygdala was demonstrated (Fox, Oler, Shackman, et al., 2015), although another study reported that amygdala functioning predictive of AT was not significantly heritable ((Oler et al., 2010) but see also (Meyer-Lindenberg, 2010) for a commentary). Together, the studies reviewed provide proof for genetic influences on amygdala functioning. However, they also illustrate that many genetic variations are likely to interact in constituting the risk for anxiety.

Criterion 4

To the best of our knowledge, no study has investigated amygdala functioning in SAD patients and their relatives at the same time. However, results from a recent high-risk study support the assumption that the SAD-related alterations in the limbic system are also found in family members of SAD patients. Children who had at least one parent with SAD ($n = 20$) showed hyperreactivity of limbic regions in response to emotional stimuli when compared to normal-risk children (Christensen, Van Ameringen, & Hall, 2015).

Taken together, the studies reviewed suggest that amygdala function and functional connectivity meet the endophenotype criterion of association with SAD. Furthermore, there is support for amygdala functioning and connectivity as relatively stable, trait-like characteristics underlying the vulnerability to SAD. In addition, several lines of evidence

provide evidence for genetic influences on amygdala functioning, while the familial co-segregation warrants more attention in future studies.

Medial prefrontal cortex: function

A core characteristic of SAD is the fear of being negatively evaluated by others (American Psychiatric Association, 2013). It is hypothesized that biases in information processing, such as the tendency to interpret ambiguous social events as negative, distorted self-referential processing, and increased attention to negative responses, play an important role in the development and maintainance of this component of SAD (Clark & McManus, 2002; Spurr & Stopa, 2002). Evidence for disturbed emotional and self-related processing in SAD has been recently reviewed (Jazaieri, Morrison, Goldin, & Gross, 2014; Stein, 2015) and several studies have linked these disturbances to altered functioning of the medial prefrontal cortex (mPFC), a brain area implicated in self-referential processing and social cognition (Amodio & Frith, 2006; Northoff et al., 2006) and the conditioning and extinction of fear (Kim et al., 2011; Quirk, Garcia, & González-Lima, 2006). The mPFC can be roughly divided into two functionally heterogeneous regions: the ventral medial prefrontal cortex (vmPFC) consisting of the subgenual anterior cingulate, ventromedial prefrontal and medial orbitofrontal cortex, mainly involved in the implicit regulation of emotion, and the dorsal medial prefrontal (dmPFC) area, including supragenual anterior cingulate and medial frontal gyrus, important for the appraisal and expression of emotions (Etkin, Büchel, & Gross, 2015; Etkin, Egner, & Kalisch, 2011; Kim et al., 2011). Structural and functional studies have indicated that the mPFC has strong connections with the amygdala (Ghashghaei, Hilgetag, & Barbas, 2007), and anxiety-related changes in both mPFC responsiveness as well as in the connectivity between the mPFC and amygdala have been reported (see review by Kim et al., (2011)). Especially alterations in the function and connectivity of the vmFPC have been associated with anxiety, as this region has a pivotal role in inhibiting conditioned fear and the extincion of a fear response (Blackford & Pine, 2012), but alterations in the dmPFC in SAD have also been reported.

Criterion 1

When we focus on the disturbances in self-related and emotional processing in SAD, studies point towards mPFC hyperreactivity, in both ventral and dorsal areas. SAD patients have increased mPFC activation levels while reading stories describing unintentional social norm transgressions (Blair et al., 2010), in response to self-related comments (Blair et al., 2008; Blair, Geraci, Otero, et al., 2011), and when viewing non-threatening sad faces (Labuschagne et al., 2011). In addition, mPFC hyperreactivity is present in SAD patients during the processing of disorder-related words like 'speech', 'to blush' and 'awkward' (Boehme, Ritter, et

al., 2015). Furthermore, anxious adolescents have increased mPFC responses to faces paired with anxiety-provoking sentences (Peris & Galván, 2013). The hyperreactivity of the mPFC in SAD was confirmed in a meta-analysis (Brühl, Delsignore, et al., 2014). Together, these studies provide evidence for the contribution of the mPFC in the SAD-related interpretation biases and disturbances in self-related processing, and highlight the potential of mPFC hyperresponsiveness as an endophenotype of SAD, although more research is needed to clarify the specific functional roles of the vmPFC and the dmPFC in SAD.

Criterion 2

The trait stability of mPFC functioning has received little attention until now. Several studies investigated the relation between mPFC functioning and temperamental traits (for a review see Kennis et al. (2013), although it should be noted that only one study investigated the relation with self-referential processing and social cognition in healthy participants. Pfeifer and colleagues demonstrated that adolescents, who are generally characterized by increased social concerns, have increased mPFC reactivity during direct self-reflection when compared to adults (Pfeifer et al., 2008). Other studies investigated the relation between inhibited temperament and prefrontal functioning using non-social tasks. Boys who were socially withdrawn during childhood showed increased mPFC responsiveness when anticipating rewards at age 20 (Morgan, Shaw, & Forbes, 2015), while adults with childhood behavioral inhibition had increased activation levels in the mPFC during conflict detection (Jarcho et al., 2013), during attention control in the context of threatening emotional faces (Jarcho et al., 2014), and during anticipation of viewing fearful faces (Clauss, Avery, et al., 2014).

The relation between inhibited temperament and hyperresponsiveness of the mPFC was recently confirmed in a meta-analysis on 13 fMRI studies (Clauss et al., 2015), while research in young rhesus monkeys demonstrated a genetic correlation between orbitofrontal brain metabolism and anxious temperament (Fox, Oler, Shackman, et al., 2015). Furthermore, studies in high socially-anxious participants demonstrated mPFC hyperresponsiveness during a paradigm in which participants were asked to focus their attention on their own bodily states, thoughts, emotions and moods in a simulated social situation (Boehme, Miltner, & Straube, 2015), and while they received social feedback on their performance during a speech task (Heitmann et al., 2014). It should, however, be noted that a study on participants with self-reported subclinical social anxiety (Abraham et al., 2013) was unable to replicate the mPFC hyperresponsivess to self-referential critisism which was previously reported by (Blair et al., 2008). Nevertheless, the majority of these findings provide cautious evidence that hyperresponsiveness of the mPFC is a trait- or vulnerability marker of anxious temperament.

Criterion 3

In comparison to the number of studies investigating genetic influences on amygdala functioning, research on the mPFC is relatively scarce. To the best of our knowledge, no study has investigated the heritability of mPFC functioning in humans. Furthermore, we are not aware of studies investigating genetic influences on mPFC function specifically in SAD. However, research on healthy participants showed an effect of variation in the serotonin transporter polymorphism (*5-HTTLPR*) on the level of mPFC activation while the participants thought about their own negative personality traits, like being lazy or greedy (Ma et al., 2014), and during reflective thinking about the discrepancy between the actual and ideal self (Shi et al., 2015), suggesting that the *5-HTTLPR* polymorphism influences self-referential processing in the mPFC.

To conclude, mPFC hyperreactivity is associated with SAD. Several lines of evidence suggest that mPFC hyperreactivity could be considered a trait characteristic, although more research, for example longitudinal research on participants at high risk for developing social anxiety, is needed to establish this with more certainty. Direct evidence regarding the heritability of this aberrant mPFC functioning in SAD and data on familial co-segregation are, however, missing, although several polymorphisms have been shown to influence mPFC activation levels.

Whole-brain functional connectivity

Over the past several years, researchers have increasingly recognized that brain regions are connected and that disturbances within brain networks could influence the onset, expression and course of diseases (Fornito, Zalesky, & Breakspear, 2015; MacNamara, DiGangi, & Phan, 2016; Sylvester et al., 2012). Thus, the field has shifted from studying specific brain regions to examining brain networks.

Criterion 1

Such network-based studies showed SAD-related changes in functional brain networks, revealing changes in functional connectivity (FC) during rest (Arnold Anteraper et al., 2014; Ding et al., 2011; Geiger et al., 2016; Liao, Chen, et al., 2010; Liao, Qiu, et al., 2010; Liu et al., 2015; Pannekoek et al., 2013) as well as during task-performance (Danti et al., 2010; Gentili et al., 2009; Giménez et al., 2012; Hahn et al., 2011; Klumpp, Angstadt, & Phan, 2012). Although the findings of these studies are mixed, probably due to relatively small sample sizes and the use of different analysis methods (see review by Brühl, Delsignore, et al. (2014)), most prominent FC changes seem to be present in the default-mode network (DMN), which is involved in social cognition and self-referential processes (Gentili et al., 2009; Liao, Chen, et al., 2010); subcortical networks involving the amygdala, caudate,

pallidum and nucleus accumbens (Arnold Anteraper et al., 2014; Manning et al., 2015); and in prefrontal and orbitofrontal networks (Ding et al., 2011). Based on the accumulated evidence to date, alterations in FC in SAD are likely. However, more research in bigger samples and with standardized methods is needed to establish the direction of SAD-related changes in FC.

Criterion 2

Several studies on healthy participants investigated the relation between FC and temperamental traits. Resting-state connectivity between the amygdala and cingulate cortex, as well as intrinsic connectivity in the DMN, the dorsal attention network, the executive control network and salience network, are influenced by trait 'social inhibition' (Blackford et al., 2014), while a recent study showed that changes in intrinsic connectivity of the DMN are already present in children (age 9 - 12 y) who are at temperamental high risk for developing social anxiety (Taber-Thomas, Morales, Hillary, & Pérez-Edgar, 2016). Furthermore, the personality trait 'extraversion' is associated with changes in whole-brain functioning connectivity (Adelstein et al., 2011; Gao et al., 2013; Lei, Zhao, & Chen, 2013), while other studies showed that individual scores of trait 'harm avoidance' (Markett et al., 2013) and trait levels of social anxiety in healthy participants (Gentili et al., 2015) moderate resting-state functional connectivity. There is also evidence for state-influences on FC: state anxiety in healthy participants correlates with resting-state amygdala-insula FC (Baur, Hänggi, et al., 2013), while a recent study on a large, population-based sample (n = 587) showed that FC measures are influenced by both stable, trait-like characteristics, as well as by state-dependent aspects (Geerligs, Rubinov, Cam-Can, & Henson, 2015) – for a review see Dubois (2016).

A couple of studies investigated the relation between the state level of social anxiety symptoms and FC measures, with mixed findings. Pannekoek and colleagues reported differences in resting-state FC in limbic and salience networks between healthy participants and SAD patients, but did not find a relationship between the level of social anxiety symptoms and FC in the patient group (Pannekoek et al., 2013). This supports the idea that FC in SAD is a trait characteristic. However, other studies reported a relationship between social anxiety symptom severity and FC (Dodhia et al., 2014; Liao, Chen, et al., 2010), and an effect of a single dose of oxytocin on resting-state amygdala-frontal connectivity in SAD patients (Dodhia et al., 2014). Future studies using standardized analysis methods should therefore investigate whether changes in FC are a trait characteristic of SAD, for example by investigating whether within-subject changes in social anxiety levels alter FC characteristics, and by examining whether changes in FC are already present in individuals at high risk for developing SAD. Mega-analyses, in which researchers combine resting-state data sets in order to maximize statistical power, could also be beneficial in examining FC changes in SAD.

Criterion 3

There is ample evidence for genetic influences on functional brain networks (Fornito et al., 2011; Glahn et al., 2010; Sinclair et al., 2015; Thompson, Ge, Glahn, Jahanshad, & Nichols, 2013). Recently, a set of 136 genes influencing FC has been identified (Richiardi et al., 2015). Furthermore, variations in the COMT genotype are shown to influence connectivity of the prefrontal cortex (Tunbridge, Farrell, Harrison, & Mackay, 2013) and the DMN (Liu et al., 2010), while variations in the serotonin receptor (*5-HT1A*) modulate activity within the DMN as well (Hahn et al., 2012). Thereby, these studies suggest that FC is at least partly heritable.

To summarize, studies have provided insight in FC changes associated with SAD. In addition, there is evidence that FC networks are generally heritable and related to trait characteristics, although these networks are influenced by state-to-state variations as well. More studies using standardized acquisition- and analysis methods are needed to establish which FC changes are robustly associated with the disorder. In addition, the state-independency and familial co-segregation of these changes call for further investigation.

Structural-anatomical changes

Since more than two decades, neuroimaging data have been used to investigate disorder-related changes in the structure of the brain. Although it is generally believed that the original goal of ascribing psychiatric disorders to specific brain areas is unlikely to be achieved due to the complex nature of such disorders, studies into the structural changes associated with psychopathology are useful to get insight in the neurobiological changes underlying these disorders, especially when a network approach is applied (Menon, 2011) .

Therefore, a handful of studies have investigated anatomical brain changes in SAD, examining alterations in gray matter volumes and differences in the integrity of white matter tracts, which will be reviewed separately in the following subsections.

Gray matter

Criterion 1

Results on gray matter (GM) density changes in SAD point towards alterations in subcortical regions like the amygdala and hippocampus, but it should be noted that these findings often lack consistency (reviewed by Brühl, Delsignore, et al. (2014)). For example, increased amygdala volumes have been found in patients with SAD (Machado-de-Sousa et al., 2014) and in young adults with inhibited temperament (Clauss, Seay, et al., 2014), while a recent treatment study revealed that successful CBT treatment decreased amygdala GM volume in SAD (Månsson et al., 2016). Interestingly, this treatment-related decrease in amygdala GM volume mediated the relationship between decreased neural reactivity of the amygdala and the reduction in social anxiety symptoms after treatment (Månsson et al., 2016), provid-

ing evidence for a link between structural and functional alterations. However, two other studies reported decreases in amygdala volume in SAD (Irle et al., 2010; Meng et al., 2013). These inconsistent results are probably due to the relatively small sample sizes or differences in methodology between studies (see (Montag, Reuter, Jurkiewicz, Markett, & Panksepp, 2013) for a critical review). However, findings from studies on GM changes in other brain regions are more consistent, showing decreases in GM in the orbitofrontal cortex and insula (Syal et al., 2012; Talati, Pantazatos, et al., 2013) and GM-increases in parietal regions (Brühl, Hänggi, et al., 2014; Irle et al., 2014; Talati, Pantazatos, et al., 2013; Tükel et al., 2015) and the temporal cortex (Frick et al., 2013b; Frick et al., 2014; Irle et al., 2014; Talati et al., 2013; Tükel et al., 2015). A recent multi-center mega-analysis on GM volumes in the largest sample to date (174 patients with SAD and 213 healthy control participants) suggests increased GM volume in the right putamen in SAD patients (Bas-Hoogendam, van Steenbergen, Pannekoek, et al., 2017), but more research in bigger samples is needed to establish which GM changes are consistently related to SAD.

Criterion 2

A couple of studies investigated the relation between GM and trait-characteristics, but their results are heterogeneous as well. To illustrate, Cherbuin and colleagues reported a positive relationship between hippocampal volume and inhibited temperament (Cherbuin et al., 2008), while another study demonstrated a negative relationship between hippocampal gray matter and anxiety-like traits (Yamasue et al., 2008). Clauss and co-workers, on the other hand, did not report changes in the hippocampus, but found an association between inhibited temperament and increased volumes of the amygdala and caudate, while further analyses showed that the increase in amygdala volume was positively associated with amygdala reactivity to neutral faces (Clauss, Seay, et al., 2014). This finding of increased amygdala volume is supported by a recent meta-analysis on the GM changes underlying personality traits linked to the vulnerability to anxiety, which revealed increased GM density in the left amygdala in individuals with high negative emotionality-related traits (Mincic, 2015). It should, however, be noted that other studies reported opposite findings, namely an association between increased amygdala volume and extraversion (Cremers et al., 2011) and positive emotionality traits (Lewis et al., 2014), or no relation between amygdala activation and extraversion (Wright et al., 2006). These contradictory findings stress again the need for further research.

Criterion 3

A considerable amount of neuroimaging studies have provided evidence for strong genetic influences on brain anatomy (for reviews see (Blokland, de Zubicaray, McMahon, & Wright, 2012; Peper, Brouwer, Boomsma, Kahn, & Hulshoff Pol, 2007; Thompson et al., 2001)). Of special interest for this review are the results of recent twin studies, indicating that the volumes

of subcortical brain structures, including the amygdala, are highly heritable from childhood on, and that the heritability estimates for these structures are stable over the years (den Braber et al., 2013; Rentería et al., 2014; Swagerman, Brouwer, Geus, Pol, & Boomsma, 2014). A GWA-study of the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium on more than 30.000 structural MRI datasets revealed common genetic variants influencing the volumes of several subcortical brain structures (Hibar et al., 2015). Furthermore, an interesting interaction between genotype, gender and amygdala volume has been found: females with two short alleles of the serotonin transporter gene *5-HTTLPR* had the highest anxiety scores and the largest amygdala volume (Cerasa et al., 2014).

Taken together, these results indicate that GM changes are, given their high heritability and their relationship with functional alterations in brain reactivity (Clauss, Seay, et al., 2014; Månsson et al., 2016), potential endophenotypes for SAD, although future research is needed to confirm which trait-like GM alterations are typically associated with SAD.

White Matter

Criterion 1

White matter (WM) density can be investigated using diffusion tensor imaging (DTI) (Thomason & Thompson, 2011). A limited number of DTI studies have investigated global WM volume in SAD: one study found a reduction in global WM, while four other studies reported no differences between SAD patients and healthy controls (see Brühl, Delsignore, and colleagues (2014) for a review). Therefore, the current state of evidence does not support global WM volume as a characteristic of SAD. However, the majority of DTI studies in SAD have focused on the integrity of one specific tract, the uncinate fasciculus (UF). This WM tract connects the amygdala with frontal cortices, including the mPFC and orbitofrontal cortex (Von Der Heide, Skipper, Klobusicky, & Olson, 2013). The mPFC is thought to regulate amygdala output and it is hypothesized that a strong connection between the amygdala and the mPFC leads to lower anxiety levels (Kim et al., 2011). SAD is repeatedly associated with reduced UF integrity (Baur et al., 2011; Baur, Brühl, et al., 2013; Phan et al., 2009). In the case of UF hypoconnectivity, control by the mPFC is likely to fail, leading to the exaggerated amygdala response in SAD (Ayling, Aghajani, Fouche, & van der Wee, 2012). This makes UF hypoconnectivity an etiologically valid candidate endophenotype of SAD. In order to get a complete view of WM changes related to SAD, future studies should examine whole brain WM integrity and investigate systematically whether other WM tracts also show SAD-related differences.

Criterion 2

Several studies investigated the relationship between anxiety-related traits and WM integrity. In line with the results of studies on SAD (Baur et al., 2011; Baur, Brühl, et al., 2013; Phan et al., 2009), two studies on healthy participants demonstrated a negative association

between trait anxiety and UF integrity (Baur, Hänggi, & Jäncke, 2012; Kim & Whalen, 2009), while another study showed a negative relation between the trait harm avoidance and WM integrity in the UF (Westlye, Bjørnebekk, Grydeland, Fjell, & Walhovd, 2011). Furthermore, reduced WM integrity of the UF was found in unmedicated preadolescent children (age 8 - 12) with anxiety disorders, suggesting that WM alterations are not caused by illness chronicity or medication use, but play a role in the pathogenesis (Tromp et al., 2015). Together, these results strengthen the idea that reduced WM integrity of the UF is a state-independent characteristic of (social) anxiety.

Criterion 3

Multiple DTI studies have indicated that WM brain characteristics are heritable (Blokland et al., 2012; Bohlken et al., 2014; Shen et al., 2014), and a meta-analysis showed that the UF has a high heritability estimate of 0.7 (Kochunov et al., 2014). This was confirmed by a recent twin-study, reporting that genetic factors explained 64 - 80 % of variance in UF microstructure (Budisavljevic et al., 2016). More specifically, structural integrity of the UF is influenced by genetic variations in the brain-derived neurotrophic factor (*BDNF*) (Carlson, Cha, Harmon-Jones, Mujica-Parodi, & Hajcak, 2014) and the *5-HTTLPR* genotype (Klucken et al., 2015; Pacheco et al., 2009).

Concluding, reduced integrity of the UF meets the endophenotype criteria of association with SAD, trait-stability and heritability. Whether this structural brain alteration and social anxiety co-segregate within families has not been examined.

Discussion

Evidence for candidate MRI endophenotypes of SAD

Here, we reviewed empirical evidence for several candidate neurobiological endophenotypes of social anxiety. Endophenotypes as measurable characteristics that are related to the disorder and reflective of genetically-based disease mechanisms. We focused on MRI measurements, and candidate endophenotypes included: function and functional connectivity of the amygdala, function of the medial prefrontal cortex (mPFC), changes in whole-brain functional connectivity (FC) and structural-anatomical alterations. Results are summarized in *Table 2.2*. Not surprisingly, given the selection of candidate endophenotypes based on studies on SAD as reviewed by Brühl and colleagues (2014a), we found strongest evidence for all candidate endophenotypes for the first endophenotype criterion, the association with the disorder. Evidence for the other endophenotype criteria (being a trait-characteristic; being heritable; and co-segregation with the illness within families) was, however, more suggestive than definitive, given the fact that direct research on these criteria is still scarce. However, available evidence from other lines of research provides circumstantial evidence

for the potential of these candidate endophenotypes of SAD. For example, studies provided evidence that amygdala-hyperreactivity and FC measures are influenced by stable trait characteristics such as inhibited temperament. Furthermore, studies on healthy participants indicate that reactivity of the amygdala and the mPFC, the strength of FC in several brain networks, changes in GM and the integrity of a specific white matter tract associated with SAD, the UF, are influenced by genetic variations. These findings highlight the potential of these neuroimaging markers as candidate endophenotypes of SAD.

Table 2.2 Evidence for candidate MRI endophenotypes of social anxiety disorder.

	Associated with SAD	Trait characteristic	Heritable	Co-segregation with illness within families
Amygdala				
Hyperreactivity and changes in functional connectivity	***	***, although also evidence for state-influences	Not directly investigated; however, genetic influences have been shown ***	*
Medial prefrontal cortex				
Hyperreactivity	***	***	Not directly investigated; however, genetic influences have been shown *	TBI
Whole-brain functional connectivity (FC)				
Altered FC during rest	***	***, although also evidence for state-influences	Not directly investigated; however, genetic influences have been shown ***	TBI
Altered FC during task performance	**	TBI	TBI	TBI
Structural-anatomical changes				
GM changes	***	**, although also evidence for state-influences	***	TBI
WM changes: integrity UF	**	**	***	TBI

Abbreviations
GM: gray matter; SAD: social anxiety disorder; TBI: to be investigated; UF: uncinate fasciculus; WM: white matter.

Footnotes
*: Evidence from 1-2 independent studies.
**: Evidence from 3-4 independent studies.
***: Evidence from ≥ 5 independent studies or a meta-analysis.

Directions for future research and outstanding questions

The reviewed evidence in favour of considering these characteristics as candidate endophenotypes of SAD is still circumstantial. To directly investigate the *heritability* and familial *co-segregation of candidate endophenotypes,* as well as their *trait-stability* (criterion 2, 3 and 4), longitudinal multiplex family studies involving patients with SAD and their family members, preferably from multiple generations, are the most optimal approach (Cannon & Keller, 2006; DeLisi, 2016). We feel the evidence summarized here provides a solid empirical background to perform such labour- and cost intensive studies, which extend the present studies comparing SAD patients and healthy control participants. To the best of our knowledge, the Leiden Family Lab study on Social Anxiety Disorder is the first comprehensive study aimed to establish neuroimaging endophenotypes of SAD. In this study, patients with SAD, their siblings and children, as well as the partners of each family member, are investigated (total sample size 134 participants of two generations, including 19 SAD patients; MRI sample size 114 participants; age range participants 8.9 – 61.5 y; see pre-registration of this study in (Bas-Hoogendam et al., 2014a)). The data are presently analyzed.

Two other outstanding issues where the endophenotype-field needs to decide upon concern the criteria for defining endophenotypes. First of all, the criterion of trait-stability or state-independency warrants more attention. An open question is, for example, whether endophenotypes could change as a result of a successful treatment. We speculate that the degree of expression of a certain endophenotype could be altered as a result of an intervention, but we hypothesize that, based on the genetic basis of the endophenotype, the endophenotype could still be detected in successfully treated patients when compared to healthy control participants without the endophenotype.

Another open question involves the specificity of endophenotypes for a particular disorder. Although the candidate endophenotypes discussed in this review are in general strongly associated with SAD, several of these characteristics are also related to other anxiety and mood disorders. For example, amygdala hyperreactvity in response to facial expressions has been found in patients with posttraumatic stress disorder (Shin et al., 2005), in participants at high risk for developing anxiety and depression (Wolfensberger, Veltman, Hoogendijk, Boomsma, & de Geus, 2008) and in patients with generalized anxiety disorder (GAD) and panic disorder (Fonzo et al., 2015); FC changes in the default mode network are demonstrated in several neuropsychiatric disorders, including depression (Whitfield-Gabrieli & Ford, 2012); alterations in mPFC functioning related to self-referential processing have been reported in patients with major depressive disorder (Nejad, Fossati, & Lemogne, 2013), while decreased white matter integrity of the UF was also present in GAD patients (Tromp et al., 2012). These findings raise the question whether these changes could serve as endophenotypes for SAD, or are rather reflective of endophenotypes for anxiety and mood disorders in general. We argue, based on the argumentation proposed by Cannon and Keller (2006), that specificity is not a prerequisite for an endophenotype. Given the fact that

several anxiety and mood disorders often run together within families (Hettema, Neale, & Kendler, 2001; Sharma, Powers, Bradley, & Ressler, 2016; Smoller, Block, & Young, 2009), it is possible that certain endophenotypes affect more than one disorder. Discovering these endophenotypes could even be helpful in unraveling the shared genetic background of these disorders (Bearden & Freimer, 2006; Cannon & Keller, 2006; Puls & Gallinat, 2008).

Methodological considerations of the present review

Given the paucity of studies examining directly whether neurobiological characteristics of SAD meet the criteria for endophenotypes (except for multiple studies on the *association with the disorder*, criterion 1), two important methodological considerations with respect to the present review should be made. First, we can not exclude that studies on SAD endophenotypes have been performed, but were not published due to negative results (i.e. a publication bias). However, we think the lack of longitudinal and family studies on SAD is primarily due to the fact that such studies are time- and cost intensive, and are hard to perform given the inherent characteristic of SAD patients to avoid attention to their impairments because they are ashamed of or underestimate their condition (Dingemans et al., 2001; Fehm et al., 2005; Ruscio et al., 2008; Stein & Stein, 2008; Wittchen & Fehm, 2003).

Second, because of the limited number of studies on neurobiological endophenotypes of SAD, the present review is a narrative rather than a systematic review. By describing results from studies which investigated evidence in relation to endophenotype criteria in multiple, related fields of research, we aimed to illustrate the endophenotype criteria using key examples from, for example, research on healthy participants with certain personality traits, and from animal research. This approach was also used in recent reviews on endophenotypes of major depressive disorder (Goldstein & Klein, 2014; Hasler & Northoff, 2011), and suited the aim of the present review, which was to explore the usefulness of endophenotypes in studying the development of SAD and to discuss the way the endophenotype approach can be applied to the field. Although we tried our best to be comprehensive, by including studies who reported results which were not in favour of certain characteristics as being endophenotypes of SAD and null findings as well, the fact that we were not able to systematically review evidence for SAD endophenotypes is a potential limitation of the present work.

Conclusions

Endophenotypes are measurable characteristics that are related to complex psychiatric disorders and reflective of genetically-based disease mechanisms. In this review, we evaluated the usefulness of endophenotypes and summarized evidence in support of neuroimagingendophenotypes of social anxiety disorder (SAD). Results are promising, but they also stress the need for further research, especially using longitudinal family studies, to assess the trait-stability of the candidate endophenotypes and the co-segregation of the endophenotype with the disorder. In addition, we pinpointed outstanding questions for the field.

Based on the circumstantial evidence already available to date, we feel neuroimaging studies have great potential to detect endophenotypes of SAD. These endophenotypes could be especially valuable in giving more insight into the mechanisms leading to this complex psychiatric disorder, which in turn provides clues for better preventive interventions and more effective treatments. Therefore, we strongly urge the need for future research specifically aimed at establishing neuroimaging endophenotypes of SAD.