The Isolated Amygdala

State and Trait Effects in Social Anxiety

Vormgeving en opmaak: Roel Mostert
Druk: Thijsen Media Group

ISBN 978-90-9027486-7

the ISOLATED AMYGDALA

State and Trait Effects in Social Anxiety

Proefschrift ter verkrijging van de graad van doctor aan de Radboud Universiteit Nijmegen op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann, volgens besluit van het college van decanen in het openbaar te verdedigen op maandag 29 April 2013 om 13.30 uur precies

door
Hendrik Roelofs Cremers
geboren op 5 September 1981
te Groningen

Promotoren:

Prof. dr. K. Roelofs
Prof. dr. P. Spinhoven (Universiteit Leiden)
Prof. dr. S.A.R.B Rombouts (Universiteit Leiden)

Manuscriptcommissie:

Prof. dr. A.R. Cools
Prof. dr. E.S. Becker
Prof. dr. B.M. Elzinga (Universiteit Leiden)

Index

Chapter 1

General Introduction

Individuals vary in the degree to which they perceive, endure and respond to different situational demands, such as stressful social situations. Personality theories aim to capture such individual differences in the experience of affect. Personality characteristics are also related to the likelihood of the development of anxiety disorders. In the context of social interactions, the extreme fear of the scrutiny of others has been differentiated for 40 years from other specific fears or agoraphobia and recognized as Social Anxiety Disorder (*Diagnostic & Statistical Manual for mental disorders* -DSM-IV-, American Psychiatric Association, 1994; Stein & Stein, 2008). This thesis presents research that investigates the neurobiology of SAD, and personality factors that are associated with its development.

1.1 Social Anxiety Disorder

Social anxiety disorder (SAD) is characterized by a persistent fear of one or more performance situations with exposure to unfamiliar people (DSM-IV, American Psychiatric Association, 1994). A person with SAD fears that he or she will act in a way that will be humiliating or embarrassing, and exposure to the feared situations almost invariably provokes anxiety, which can take the form of panic attacks (Stein & Stein, 2008). Social situations are either avoided or endured with intense anxiety or distress (Stein & Stein, 2008). The diagnosis of SAD requires that the condition interferes substantially with the person's normal routine (Stein & Stein, 2008). The lifetime prevalence rates vary between 5-12 percent depending on the inclusion criteria (Stein & Stein, 2008). Social anxiety can be treated relatively well, with both pharmacotherapy and psychotherapy (Fedoroff & Taylor, 2001; Stein & Stein, 2008). When both are directly compared, pharmacological treatment seems to have a stronger immediate result, but the effects of behavioral treatment last longer (Gelernter et al., 1991; Heimberg et al., 1998; Stein & Stein, 2008). Nonetheless, a considerable number of patients do not respond adequately to treatment (Gaston, Abbott, Rapee, & Neary, 2006; Hofmann, 2007). For example, it has been estimated that about 40-50 percent of the patients do not show clinical improvement after cognitive behavioral treatment (Eskildsen, Hougaard, & Rosenberg, 2010). Therefore new treatment is continuously being developed (Hofmann, 2010).

Much is still unknown about the pathogenesis of SAD and the factors that influence its development, course and treatment response (Kimbrel, 2008). Different perspectives or approaches (cultural, developmental, cognitive, neurobiological, genetic) are possible in studying SAD (or any psychiatric disorder), all of which can offer valuable insights (Henningsen & Kirmayer, 2000). While research into new treatments is obviously vital for improving treatment responses, insight into the more fundamental mechanisms of a disorder is crucial, besides gaining basic theoretical knowledge (e.g. Bishop, 2008; Casey et al., 2011; Perlis, 2011). For example, theoretical insight into the mechanisms of SAD, showing the importance of attention biases and performance feedback, has helped to improve standard cognitive behavioral therapy (Rapee, Gaston, & Abbott, 2009). In addition, insight into brain mechanisms underlying psychopathology is essential for the understanding of psychiatric disorders (Bullmore, Fletcher, & Jones, 2009), although much progress still has to be made (Kapur, Phillips, & Insel, 2012).

1.2 Models of personality and social anxiety

The structure of personality and its biological basis have been topic of research for several decades (Cloninger, 1986; Davidson & Irwin, 1999; Eysenck, 1967; Gray, 1978; McCrae & Costa, 1991; Zelenski & Larsen, 1999). Many trait theories commonly emphasize broad and theoretically orthogonal predispositions, which are further organized hierarchically into subcomponents (Bouchard & Loehlin, 2001; Naragon-Gainey & Watson, 2011; Watson, Clark, & Harkness, 1994). Trait theories often include at least one trait linked to "susceptibility" for positive and one for negative affect (Zelenski & Larsen, 1999). For example, Eysenck's classical approach contains the extraversion/neuroticism dimension, Gray distinguished Behavioral Activation System (BAS)/Behavioral Inhibition System (BIS), while Cloninger included Novelty Seeking/Harm Avoidance (Zelenski & Larsen, 1999). These traits are thought to reflect dimensions in an affect-circumplex: a two-dimensional space that describes emotional experience as coordinates within this space (Russell, 1980). Debate exists on exactly how these traits are oriented; for example, the BIS/BAS axes from Gray's theory are thought to be a 45 degree rotation from the Neuroticism/Extraversion dimensions from Eysenck (Zelenski & Larsen, 1999). The empirical basis for these theoretical rotations has been questioned however (Smits & Boeck, 2006), and a factor analysis has indeed shown that when questionnaire data of different trait theories are combined into one analysis, two traits adequately describe the experience of positive and negative affect across individuals (Zelenski & Larsen, 1999). The division of personality into positive affect and negative affect traits will be operationalized here as the neuroticism/extraversion distinction. While neuroticism and extraversion are also part of the "big

CHAPTER 1

five trait theories" (McCrae & Costa, 1991) and in some models form subcomponents of higher-order traits (DeYoung et al., 2010; Markon, Krueger, & Watson, 2005), here, they will be regarded as the two main personality traits directly related to the experience of affect (McCrae & Costa, 1991). In that sense, the distinction between neuroticism and extraversion also seems a useful starting point in linking personality to psychopathology (but see for example Ormel, Rosmalen, & Farmer (2004), for a critical discussion of the relevance of neuroticism).

Neuroticism is characterized by a temperamental sensitivity to painful or negative stimuli, and experiencing negative affect more frequently and/or intensely. Extraversion is a temperamental sensitivity to pleasurable stimuli (rewards) and experiencing positive affect, pride and self-confidence more frequently and/or intensely (Winter & Kuiper, 1997). Research on extraversion and neuroticism has shown that in the development of SAD, neuroticism is a vulnerability marker, and extraversion a protective factor (Bienvenu, Hettema, Neale, Prescott, & Kendler, 2007; Clark, Watson, & Mineka, 1994; Naragon-Gainey & Watson, 2011; Spinhoven et al., 2011). Neuroticism is thought to be an unspecific risk factor (i.e. it is related to many psychiatric disorders), while extraversion is perhaps more specifically related to depression and SAD (Naragon-Gainey & Watson, 2011). In addition, the study by Naragon-Gainey & Watson (2011) describes a more detailed link between neuroticism/extraversion and hierarchically lower subcomponents (see figure 1.1). For neuroticism, the most relevant subcomponents in relation to SAD are evaluation sensitivity and social concerns, while for extraversion, sociability and dominance are most strongly related to SAD (Naragon-Gainey & Watson, 2011). In line with these findings, a recent model of SAD puts great emphasis on the basic personality predisposition in the development, course and treatment of SAD (Kimbrel, 2008). Although the Kimbrel model relies on Reinforcement Sensitivity Theory (Gray's BIS/BAS axes), the relation of the BIS/BAS axes with SAD is very similar to the Neuroticism/Extraversion findings just discussed, in that BIS is regarded as a predisposing factor and BAS as a protective one for the development of SAD.

However, the factors that influence the development of SAD clearly go beyond basic personality traits, and include other elements such as genetic makeup and several environmental causes. It is of interest that personality factors have a considerable genetic component (Bouchard & Loehlin, 2001) and it has been found that the heritability of social anxiety can be explained by the heritability of personality traits (Bienvenu et al., 2007). Many different "routes" are possible in the development of SAD (principle of multifinality) and similar predisposing factors may lead to very different outcomes (principle of equifinality; Kimbrel, 2008). Personality traits constitute so-called *distal factors* in the development of SAD, where for example attention and interpretation biases (which form the basis of many cognitive models of SAD) are thought of as *proximal factors* (Kimbrel, 2008). Several cognitive models have been proposed for SAD (Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Rapee & Heimberg, 1997) and they all describe broadly similar concepts (Hoffman, 2010), including low perceived emotional control, post-event rumination, avoidance and the use of safety behaviors.

The distal and proximal factors interact in a complex manner, for example in the relation between personality, life experience, and social support. It is well-known that childhood traumatic experience increases the risk of anxiety disorders and heightened responsiveness of the stress system (Elzinga, Spinhoven, Berretty, de Jong, & Roelofs, 2010; Heim & Nemeroff, 2001; Kaufman, Plotsky, Nemeroff, & Charney, 2000; Spinhoven et al., 2010), but adequate caregiving moderates this trauma-stress/anxiety link (Heim & Nemeroff, 2001). Moreover, attachment security decreases the relation between behavioral inhibition and stress reactivity (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). On the other hand, maternal overprotection combined with high behavioral inhibition are risk factors for developing social anxiety (Lewis-Morrarty et al., 2012). Another study showed that experiencing uncontrollable events increases the relation between behavioral inhibition and anxiety (Chorpita & Barlow, 1998). Moderate (non-traumatic) stressful situations might be habituating (decreasing responsiveness over time) to one individual, but sensitizing (increasing responsiveness over time) to another, depending on such factors as social support, personality traits and gender. For example, one study showed that high social support was associated with habituation of cardiovascular responses to repeated stress exposure in woman, but not in men (Hughes, 2007). In addition, a recent study emphasizes the relation between sociability factors and neuroticism in the developmental trajectories of pediatric social anxiety (Miers, Blöte, de Rooij, Bokhorst, & Westenberg, 2012). Insight into the neurobiology of important processes in the development of SAD and the relation to individual differences in personality may help to explain the mechanisms by which social anxiety can develop. Two of these processes, emotion regulation and motivational reward/punishment balance, and their underlying neurobiological mechanisms, are crucial in anxiety disorders (Degnan & Fox, 2007) and will be the topic of the current thesis.

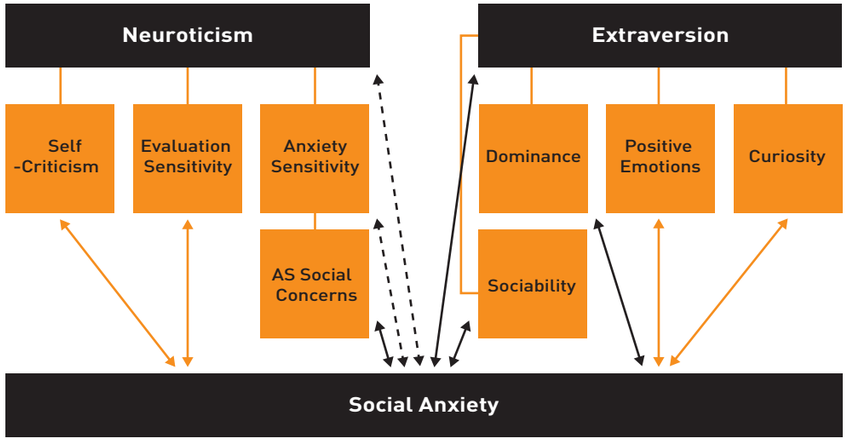

Figure 1.1 Schematic representation of social anxiety's associations with (hierarchically-arranged) personality traits
Dashed lines with arrows indicate low specificity to social anxiety (i.e., the trait is more strongly related to several other disorders); solid orange lines with arrows indicate moderate specificity (i.e., the trait is related equally to one or two other disorders); thick solid black lines with arrows indicate strong specificity (i.e., the trait is more strongly related to social anxiety than to other disorders). All associations of social anxiety with neuroticism and its lower-order factors are in the positive direction; all associations of social anxiety with extraversion and its lower-order factors are negative. AS = anxiety sensitivity. This figure is reproduced with permission from (Naragon-Gainey & Watson, 2011).

1.3 Neurobiological Circuits

In the broadest sense, the psychological processes that are studied in the current thesis are part of the "mental trilogy": cognition, emotion and motivation (LeDoux, 2002). The brain-basis for interaction between cognition and emotion/motivation is the communication of cortical and subcortical brain regions. Basic research has elucidated these cortical-subcortical neurobiological pathways in certain detail, and they can be described as circuits of brain regions. Here, the focus will be on two of these circuits: the *emotion circuit* (centered around the amygdala) and the *motivation circuit* (centered around the ventral striatum), see figure 1.2. These circuitries have been linked to *resilience* to adverse and stressful events and the development of affective disorders (Feder, Nestler, & Charney, 2009).

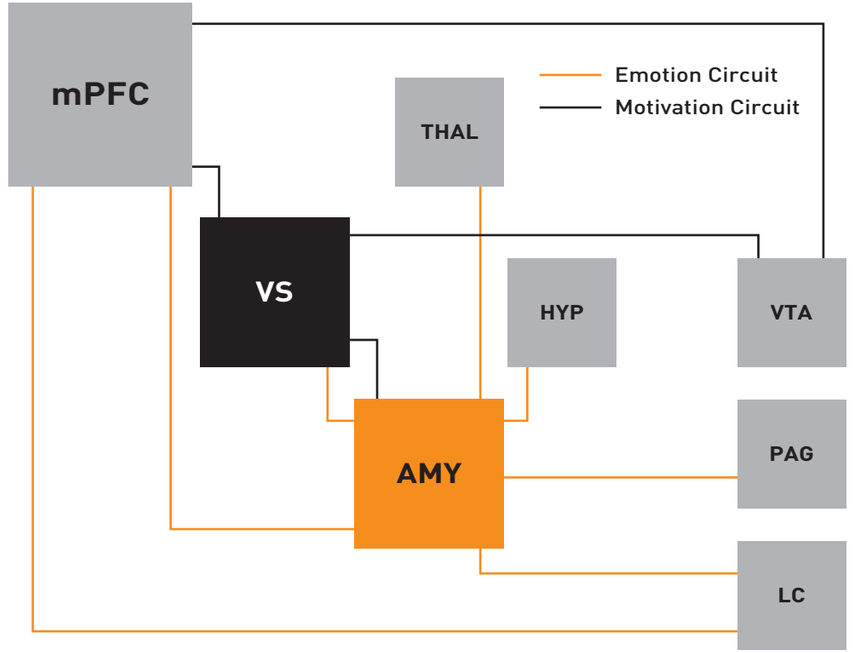

Figure 1.2 A schematic model of brain regions involved in emotion and motivation
A schematic overview of regions involved in emotion and motivation, divided into two broad circuits (based on Feder, 2009). The emotion circuit: salient sensory information is relayed from the sensory cortex (not shown) and the thalamus to the amygdala. The amygdala is particularly important for emotion processing (learning and memory), typically studied in relation to threat or fear processing (and for example the freezing responses, for which the connections with the periaqueductal grey are important), but it is also involved in other emotions. Moreover, the amygdala is connected to regions involved in the autonomic (locus coeruleus, release of norepinephrine) and hormonal (hypothalamus, release of Corticotrophin-Releasing Hormone) responses to stress. The motivation circuit: The ventral striatum receives dopaminergic input from the ventral tegmental area, in response to impeding or obtained incentives. At several brain structures, both circuitries are influenced by (medial) prefrontal regions, which can exert top-down control. While these two circuits are partly separable, anatomically and functionally they also overlap, especially at the connection between the amygdala and ventral striatum. Note that this is a highly simplified model; many other connections exist which are not shown, and in addition many of the presented regions consist of several subregions, characterized by specific patterns of connectivity and functioning. Amy, amygdala; Hyp, Hypothalamus; LC, locus coeruleus; MPFC, medial prefrontal cortex; Thal, Thalamus; VS, ventral striatum; VTA, ventral tegmental area; PAG, periaqueductal grey.

1.3.1 The amygdala and the emotion circuit

The amygdala is a core region involved in emotion processing (LeDoux, 1998; 2000). Although originally, the function of the amygdala was mainly related to the emotion of fear, due to its evident importance in fear conditioning (LeDoux, 2000), currently its role is perceived much broader. The amygdala is critical in detecting salient information in the surroundings, and it is linked to the processing of salient or ambiguous stimuli (Davis & Whalen, 2001; Whalen, 2007). In humans, there is an abundance of evidence for the importance of the amygdala in face processing (Costafreda, Brammer, David, & Fu, 2008), although perhaps more generally, to several facial expressions than to any expression (e.g. fear or anger) specifically (Costafreda et al., 2008; Fitzgerald, Angstadt, Jelsone, Nathan, & Phan, 2006). Anatomically, several subnuclei in the amygdala can be distinguished, which have distinct patterns of connections (Price, 2003). The basolateral Amygdala (BLA) receives sensory input from many cortical sources and from the thalamus (Price, 2003). The central nucleus of the amygdala (CeA) projects to numerous cortical and subcortical regions. Important connections include the link to the (ventral) periaquaductal grey (PAG), which is essential for freeze responses to threat (Fanselow, 1994). Other essential connections include the hypothalamus (Price, 2003), involved in the release of the stress hormone Corticotrophin Releasing Hormone (CRH), and the locus coeruleus (LC), a brain stem nucleus, important for noradrenaline release during stress (Valentino & Van Bockstaele, 2008). Through its connections with the Hypothalamus and LC, the amygdala plays an integrated role in both the endocrine and autonomic nervous system reactions in response to stress (Arnsten, 2009; Joëls & Baram, 2009; Ulrich-Lai & Herman, 2009). However, the exact role of the amygdala in prolonged states of social stress is still topic of debate. For example, it is unclear whether and how the amygdala is controlled during stress, and amygdala activation as well as amygdala deactivation during social stress have been found (Pruessner et al., 2008; Wager et al., 2009).

1.3.2 The ventral striatum and the motivation circuit

The motivation circuit is a well-described set of brain regions in the brainstem, striatum and medial prefrontal cortex (mPFC) that rely strongly on mesolimbic dopaminergic activity (Haber & Knutson, 2010). The ventral tegmental area (VTA) is the main brainstem source of dopamine and is connected to regions of the ventral striatum. The ventral striatum includes the nucleus accumbens, and ventral parts of the putamen and caudate nucleus. Functionally, the ventral and dorsal striatum are argued to be best distinguished by their connectivity to the medial Orbitofrontal Cortex/Anterior Cingulate Cortex and dorsolateral Prefrontal Cortex respectively (Haber & Knutson, 2010). Despite the extensive amount of research, considerable debate is still going on about the type of functions related to the motivation circuit (Salamone, 2009). Dopamine serves to promote complex functions such as reinforcement learning (Cools, Nakamura, & Daw, 2011; Salamone, 2009; Schultz, 2004) and the anticipation of a reward is also strongly related to dopaminergic functioning in this circuit. Hence, the set of regions is often referred to as the reward circuit (Knutson & Greer, 2008). However, an accumulating amount of studies have now shown that the selective focus on rewards is incomplete. A number of recent reports has shown that different dopamine neurons are involved in *motivational valence* (DA neurons that are responsive only to impeding rewards) and *motivational salience* (DA neurons which respond to both impeding reward and punishment, as far as they can actively be avoided; Bromberg-Martin, Matsumoto, & Hikosaka, 2010; Matsumoto & Hikosaka, 2009). These findings complement previous notions of the importance of including aversive motivation in the role of the striatum (Salamone, 1994; 2009). Studies in humans have further underscored this broader view of striatal (dopaminergic) activity. For example, the striatum was shown to be involved in fear conditioning and avoidance learning (Delgado, Li, Schiller, & Phelps, 2008a).

1.3.3 The prefrontal cortex and emotion regulation

The prefrontal cortex (PFC) is involved in many higher-order cognitive functions (working memory, executive control, task-switching generally referred to as cognitive control; Miller & Cohen, 2001). In addition, in the domain of emotions, the PFC is crucial for modifying emotion responses (Ochsner & Gross, 2005). A recent meta-analysis has identified a very broad set of prefrontal, as well as parietal regions involved in emotion regulatory processes (Diekhof, Geier, Falkai, & Gruber, 2011). While some regions are related to specific types of emotion regulation, the ventromedial PFC is involved in various kinds of emotion regulation (fear conditioning, placebo control, cognitive regulation of emotion). The prefrontal cortex has many connections to regions part of the emotion and motivation circuits and can hence exert a regulatory role over various processes in these regions. For example, the amygdala has major cortical connections, and the connections with the ventromedial PFC (vmPFC) are crucial for the inhibition of the amygdala during fear extinction

(Quirk & Mueller, 2007). However, the amygdala is also more widely connected to other cortical areas such as the dorsomedial PFC (DMPFC) and Posterior Cingulate Cortex (Stein et al., 2007). Research in humans has shown similarities between cortical regulation of the amygdala during fear extinction and instructed emotion regulation (Delgado, Nearing, LeDoux, & Phelps, 2008b; Hartley & Phelps, 2009; Quirk & Beer, 2006). This observation may suggest that the human capacity to voluntarily regulate emotional responses is mediated by phylogenetically older fear circuits. It is generally hypothesized that reduction of this prefrontal emotion regulatory capacity may form the basis of various anxiety disorders (Bishop, 2007; Hartley & Phelps, 2009; Kim et al., 2011).

1.3.4 Integration of neural emotion and motivation circuitries

Emotional and motivational functions have been closely linked in various scientific perspectives (e.g. theoretical, neuro-anatomical). In several emotion theories, reward and punishment processing are central components, while decision-making ("motivation") theories highlight the role of emotional value (Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994; Knutson & Greer, 2008; Loewenstein, Weber, Hsee, & Welch, 2001; Rolls, 1990). For instance, in accordance with the concept of loss aversion, people are generally more sensitive to the possibility of losing objects or money than they are to the possibility of gaining those objects or amounts of money (Kahneman & Tversky, 1984; Tom, Fox, Trepel, & Poldrack, 2007). Another example is the somatic marker hypothesis, which states that during complex risky decisions, somatic markers (physiological emotional states) guide the motivational decision-making process (Damasio, 1996).

Similarly, research data from different levels and domains of brain research suggest that the emotion and motivation circuitries are closely linked. For example, it has been shown that the amygdala plays an important role in reward processing (Baxter & Murray, 2002). Moreover, acute stress leads to an increase in dopamine (Arnsten, 2009; Kienast et al., 2008; Mizrahi, 2010; Pruessner, Champagne, Meaney, & Dagher, 2004), stress induction disrupts PFC function during reward anticipation (Ossewaarde et al., 2011), while cortisol administration increases risk taking (Putman, Antypa, Crysovergi, & van der Does, 2010). While acute stress may increase dopamine and reward sensitivity, prolonged stress may reduce reward sensitivity (Ossewaarde et al., 2011). Indeed, several affective disorders, such as depression, have been associated with reduced reward sensitivity (Feder et al., 2009).

On the neurotransmitter level, complex interactions (both opposing and complementary) between the serotonergic and dopaminergic systems (Boureau & Dayan, 2011; Cools et al., 2011) and the noradrenergic and dopaminergic systems (Aston-Jones & Cohen, 2005) have been highlighted. On the brain system level, one study showed that the emotion and motivation circuitries are differentially involved in cognitive emotion generation and regulation, which involved lPFC-amygdala and lPFC-Nacc pathways respectively (Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). The interactions between different subsystems of the neural motivation and emotion circuitries are complex therefore, and much remains to be learned. Because of their proposed common importance in stress resilience (Feder et al., 2009), the functioning of these neural circuitries seems essential to understanding the neurobiology of anxiety disorders.

1.3.5 Neuroimaging research in SAD

Initial functionl Magnetic Resonance Imaging (fMRI; see box 1) studies focused on amygdala responses to negative (angry/fearful) faces, and amygdala hyperactivation has been shown in SAD (Etkin & Wager, 2007). Exaggerated amygdala responses were also found during speaking in public (Tillfors, Furmark, Marteinsdottir, & Fredrikson, 2002; Tillfors et al., 2001), a prototypical example of the situation SAD patients fear most. Recent studies have begun aiming at other more specific symptom-relevant aspects of SAD, like self-relevant praise or critique (Blair et al., 2008), social norm processing (Blair et al., 2010), peer evaluation (Guyer et al., 2008), and the reappraisal of negative self-beliefs (Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009). The ventral striatum and the motivation circuit have also been implicated in SAD. For example, several studies have shown alterations in dopaminergic activity in SAD. However, both increases and decreases were found (Freitas-Ferrari et al., 2010; van der Wee et al., 2008). One fMRI study has linked differences in striatal activity to implicit learning processes (Sareen et al., 2007) and other research in (pediatric) social anxiety have shown a general increase in striatal activity both for reward and punishment avoidance (Guyer et al., 2012; 2006). Much remains unknown about how social anxiety is linked to connectivity and activity within (and between) these neural emotion and motivation circuitries.

1.4 Aim and outline of the thesis

The aim of the current thesis is to investigate neurobiological mechanisms in social anxiety. The focus is on two broad sets of interconnected regions, the neural emotion and motivation circuit-

ries, and their relation to state and trait aspects of SAD. The first subgoal is to address the neural structural and functional correlates of the affect-related personality traits of extraversion and neuroticism. The second sub goal is to study symptom-relevant processes in social anxiety: the motivational balance to obtain a social reward or avoid a social punishment, and the processing of stress-inducing social evaluative threat. In addition, we investigate how differences between SAD and controls in brain connectivity during social stress processing are already present in healthy controls depending on variation in personality traits. Lastly, the link between the neural mechanism of motivational preference (reward and punishment sensitivity) and social stress processing will be explored.

Chapters 2 and 3 will address individual differences in neuroticism and extraversion in healthy controls. These studies are part of the Netherlands Study on Depression and Anxiety (NESDA; Penninx et al., 2008). While there is some evidence for a modulatory role of neuroticism on task-induced brain *activity*, much remains unknown about how neuroticism is linked to brain *connectivity*, especially the crucial coupling of the amygdala and the prefrontal cortex.
In **Chapter 2,** this relation between neuroticism and functional connectivity of the amygdala and cortical regions during the processing of negative facial expressions is investigated.
Chapter 3 will test whether individual differences in neuroticism as well as in extraversion are related to brain volume in regions of the emotion and motivation circuitries.

Cognitive theories conceptually link SAD to heightened punishment sensitivity; however, current neurobiological findings have not shown evidence of this. **Chapter 4** examines social reward and punishment anticipation in SAD. The hypothesis to be tested is that in SAD, the striatum is more sensitive (stronger active) when a social punishment can be avoided than when a social reward can be obtained. Severe stress during the anticipation of speaking in public is a core symptom of SAD. Connectivity between the amygdala and cortical regions is thought to be important for emotion regulation, a function that is compromised in SAD. However, it is unknown whether and how cortical–amygdala connectivity is affected during stress-inducing social evaluative threat.

Chapter 5 studies cortical-amygdala connectivity in SAD during a resting-state ("task-free") condition and when anticipating speaking in public. It is hypothesized that cortical-amygdala connectivity is related to cortical cognitive control of the amygdala, and that this regulation is diminished

in SAD during the anticipation of speaking in public. Also, the question will be addressed of whether this cortical-amygdala connectivity is a mediator in the relation between anxiety symptoms and perceived stress.

Chapter 6 investigates the relation of cortical-amygdala connectivity with neuroticism and extraversion. The same method and data of chapter 5 will be used, but now focusing on the healthy control participants. It will be tested whether neuroticism and extraversion modulate cortical-amygdala connectivity, and whether this relation is dependent on the task-phase (speech anticipation or baseline measurements).

Chapter 7 discusses some problematic (statistical) issues in fMRI research on clinical populations and other between-subject effects research. It will be argued that the combination of extremely low statistical power, high flexibility in data analyses, and the lack of direct, quantitative replication studies, impairs the interpretation of most published findings of fMRI research on clinical populations.

Chapter 8 summarizes and discusses the empirical findings. The results of the chapters on personality traits (chapters 2, 3 and 6) and SAD (chapters 4 and 5) will be discussed in more detail. In addition, the results of chapters 4 and 5 will be linked, to test whether reward/punishment sensitivity is predictive of social stress processing. Lastly, limitations and considerations for future research will be discussed.

Box 1. Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) is a powerful tool to non-invasively obtain whole brain coverage of an approximation of neural activity. fMRI is an extremely complex technique, which allows reconstruction of images from magnetic resonance properties of nuclei (see Logothetis, 2008 for an overview). The signal often measured with fMRI is the Blood Oxygen Level Dependent (BOLD) signal. BOLD-fMRI relies on the magnetic properties of oxygenated blood in the brain. An increase in brain activity will change various physiological parameters of the blood, thereby influencing the magnetic properties. This change in magnetic properties affects the measured signal, which can be quantified and reconstructed to its original location. The BOLD signal thus forms an indirect measure of brain activity. The BOLD signal is relatively slow (the signals peaks 4-8 seconds after the onset of neural activity) and hence has a poor *temporal* resolution. However, the *spatial* resolution is relatively good (in order of millimeters).

The basic approach of many fMRI studies is to have participants perform a task while lying in the scanner. This task usually consists of at least two conditions that can be compared against each other (for example viewing emotional and neutral faces). The difference in signals between the two conditions can then be interpreted as brain *activity* related to one condition over another (i.e. the emotional expression of a face). It is widely acknowledged, however, that brain functioning is not just captured by local activity within a region, but also by connectivity between different regions. Many analytic techniques have been, and continue to be, developed to capture brain *connectivity* (Smith, 2012). In addition to studying brain functioning related to external demands (a computerized tasks that has to be performed), much research is also devoted to studying the brain "at rest", the so-called "default mode" of brain functioning (Raichle et al., 2001). Brain processes at rest give valuable information about ongoing mental functioning intrinsically generated (Raichle, 2010).

Chapter 2

Neuroticism modulates amygdala – prefrontal connectivity in response to negative facial expressions.

Henk Cremers | Ramona Demenescu | André Aleman | Remco Renken
Marie-José van Tol | Nic J.A van der Wee | Dick J. Veltman | Karin Roelofs

NeuroImage, 2010, 49(1), 963–970.

2.1 Abstract

Objective: Neuroticism is associated with the experience of negative affect and the development of affective disorders. While evidence exists for a modulatory role of neuroticism on task induced brain *activity*, it is unknown how neuroticism affects brain *connectivity*, especially the crucial coupling between the amygdala and the prefrontal cortex. Here we investigate this relation between functional connectivity and personality in response to negative facial expressions.

Methods: Sixty healthy control participants, from the Netherland Study on Depression and Anxiety (NESDA), were scanned during an emotional faces gender decision task. Activity and functional amygdala connectivity (psycho-physiological interaction; PPI) related to faces of negative emotional valence (angry, fearful and sad) was compared to neutral facial expressions, while neuroticism scores were entered as a regressor.

Results: Activity for fearful compared to neutral faces in the dorsomedial prefrontal (DMPFC) cortex was positively correlated with neuroticism scores. PPI analyses revealed that right amygdala–DMPFC connectivity for angry and fearful compared to neutral faces was positively correlated with neuroticism scores. In contrast, left amygdala – anterior cingulate cortex (ACC) connectivity for angry, fearful and sad compared to neutral faces was negatively related to neuroticism levels.

Conclusions: DMPFC activity has frequently been associated with self-referential processing in social cognitive tasks. Our results therefore suggest that high neurotic participants display stronger self-referential processing in response to negative emotional faces. Secondly, in line with previous reports on ACC function, the negative correlation between amygdala – ACC connectivity and neuroticism scores might indicate that those high in neuroticism display diminished control function of the ACC over the amygdala. These connectivity patterns might be associated with vulnerability to developing affective disorders such as depression and anxiety.

2.2 Introduction

Neuroticism is a widely recognized trait in various theoretical approaches to human personality (Smits & Boeck, 2006; Zelenski & Larsen, 1999). Characteristics of this trait include a tendency to worry and to be anxious (Canli et al., 2001), and to the experience of negative affect (Larsen & Ketelaar, 1991; Robinson, Ode, Moeller, & Goetz, 2007a; Zelenski & Larsen, 1999). Neuroticism is also associated with affective disorders such as social anxiety disorder (SAD) and depression (Bienvenu et al., 2001; Clark, Watson, & Mineka, 1994).

Functional magnetic resonance imaging (fMRI) studies have provided substantial evidence for the modulatory role of individual differences in neuroticism on neural activity related to emotion processing (Canli, 2004; Hamann, 2004). Regions where activity is associated with neuroticism (and related personality traits) include the amygdala (Haas, Omura, Constable, & Canli, 2007; Reuter et al., 2004; Stein, Simmons, Feinstein, & Paulus, 2007b), the anterior cingulate cortex (ACC) (Eisenberger, Lieberman, & Satpute, 2005; Reuter et al., 2004) and the medial prefrontal cortex (Britton, Ho, Taylor, & Liberzon, 2007; Haas et al., 2007; Rubino et al., 2007). However, these regions are functionally coupled, and connectivity between amygdala and prefrontal regions is crucial for the integration between emotion and cognition (Pessoa, 2008; Stein et al., 2007a). To gain a better understanding of the neural basis of individual differences in emotion processing related to neuroticism, a focus on functional connectivity between limbic and prefrontal regions is therefore required.

Neuroticism is associated with alterations in cognitive-emotional functions such as affect regulation (Tamir, 2005) self-consciousness (Trapnell & Campbell, 1999) and self-regulation (Robinson, Ode, Wilkowski, & Amodio, 2007b). Thus, dysfunctional interactions between the amygdala and regions related to these functions, such as ventrolateral PFC (VLPFC), dorsolateral PFC (DLPFC) and ACC (cognitive control of emotion; Ochsner & Gross, 2005; Pessoa, 2008), and dorsomedial prefrontal cortex -DMPFC- (self-regulation and self-referential processing; Amodio & Frith, 2006; Northoff & Bermpohl, 2004) are likely to be specifically associated with individual differences in neuroticism.

Only recently, fMRI studies have started to investigate personality-associated differences in functional connectivity during emotion processing. Whereas some of these studies focused on traits related to positive affect (Haas, Omura, Amin, Constable, & Canli, 2006; Passamonti et al., 2008), one study reported *trait anxiety* differences in amygdala-ACC coupling (Kienast et al., 2008). A mood induction study during Positron Emission Tomography (PET) showed that neuroticism is associated with changes in subgenual cingulate coupling with prefrontal regions during mood

induction, possibly reflecting a susceptibility marker for depression (Keightley et al., 2003). Despite these initial findings, to the best of our knowledge, no study has systematically addressed the question on how individual differences in neuroticism are associated with amygdala – prefrontal cortex connectivity for various negative emotional facial expressions.

To investigate the modulatory role of neuroticism on amygdala – prefrontal cortex connectivity during emotion processing, we applied a standardized face-processing paradigm with different negative emotional facial expressions (angry, fearful and sad) in a large subject sample. This sample represented the healthy control subjects as part of the Netherlands Study on Depression and Anxiety (NESDA; Penninx et al., 2008). We hypothesize that *activity* in the medial PFC, ACC and the amygdala is associated with neuroticism scores when processing negative as compared to neutral facial expressions. We also hypothesize that *connectivity,* between the amygdala on one hand and the lateral and medial prefrontal regions and the ACC on the other, vary with individual differences in neuroticism.

2.3 Methods

Participants

Sixty healthy participants were selected from the general population (mean age = 39.9, range 21-56, 37 females). Participants were recruited as healthy control participants in a large multi-center cohort study, the Netherlands Study of Depression and Anxiety (NESDA). Participants were tested at the Amsterdam Medical Center (AMC), Leiden University Medical Center (LUMC) and University Medical Center Groningen (UMCG). The exclusion criteria for these healthy participants were (1) a lifetime diagnosis of DSM axis I and/or axis II disorders, psychotic disorder or dementia, (2) current alcohol or substance abuse, (3) a history of seizure or head injury, (4) current use of beta-blockers medication, (5) hypertension (high blood pressure) 180/130 mm Hg, (6) more than 5 cigarettes smoked per day, (7) older than 57 years and (8) MRI incompatible implants or tattoos. During the preliminary analysis, 4 participants were excluded because of head movement artifacts. Written informed consent from each participant was obtained prior to the scanning session. The study was approved by the Ethical Review Boards of each participating center.

Personality scores

To asses personality traits, all participants completed the NEO Five Factor Inventory (Costa & McCrea, 1992). This questionnaire consists of 60 items and measures five different personality traits: neuroticism, extraversion, openness, agreeableness and conscientiousness. Of these traits, neuroticism and extraversion are most closely related to emotion processing and alterations in neural activity (Canli, 2004). Examples of the neuroticism questions include 'I often feel less then other people', 'I often feel nervous and tense'.

Experimental design

Color photographs of faces depicting angry, fearful, sad, happy, and neutral facial expressions were presented together with scrambled faces in an event-related design. Photographs were selected from the Karolinska Directed Emotional Faces System (Lundqvist, Flykt, & Ohman, 1998) representing standardized facial expressions of emotions presented by amateur actors. Twenty-four faces were selected for each of the five facial expressions, comprising of 12 female and 12 male faces, and 80 scrambled faces. A total number of 200 photographs were presented pseudorandomly, such that there were maximally two faces presented before the presentation of a scrambled face, and there were no repetitions of the same emotional expressions. Each photograph was presented on the screen for 2.5 s, with an inter-stimulus interval (black screen) varied between 0.5 and 1.5 s (jitter). The total duration of the task was 747 s. The experimental paradigm was presented using E-prime software (Psychological Software Tools, Pittsburgh, PA, USA). Images were projected onto a translucent screen at the end of the scanner bed, visible via a mirror above the participant's head. Participants were instructed to indicate the gender by pressing one of two buttons of two magnet-compatible button boxes with the index finger of the left or right hand. During the presentation of scrambled faces, participants had to press left or right buttons in conformity with the instructions present on the screen, indicating either left or right by an arrow. Responses and reaction times were recorded.

Image acquisition

Images were acquired on a Philips Intera 3T MR-scanner. A sense-8 (UMCG and LUMC) and a sense-6 (AMC) channel head coil was used for radio frequency transmission and reception. For each subject a

series of echo planar imaging (EPI) -sensitive to the blood oxygenation level dependent effect- volumes were obtained, entailing a T2*-weighted gradient echo sequence (repetition time [TR] = 2300 ms, echo time [TE] = 28.0 ms at UMCG and TE = 30.0 ms at AMC and LUMC, flip angle 90 using axial whole-brain acquisition, with an interleaved slice acquisition order. The interslice gap was 0 mm and the plane thickness was 3 mm. The matrix sizes were: 64x64 voxels at UMCG and 96 x 96 voxels at AMC and LUMC. The EPIs were acquired at 39 slices at UMCG and 35 slices at AMC and LUMC. The in-plane resolution was 3 x 3 mm at UMCG and 2.29x2.29 mm at AMC and LUMC. The axial images were acquired parallel to the anterior-posterior commissure plane. Functional data comprising 310 volumes were obtained per subject. A T1-weighted anatomical MRI was also acquired for each subject (TR = 9 ms, TE = 3.5 ms, matrix size 256x256).

Analysis

Preprocessing

Functional data were preprocessed and analyzed using the statistical parametric mapping software package (SPM5, http://www.fil.ion.ucl.ac.uk) implemented in Matlab 7.2 (The MathWorks Inc., http://www.mathworks.com).

The EPI volumes were reoriented in respect to the anterior commissure selected on the first volume. Time series were corrected for differences in slice acquisition times. The reference slice was 39 at UMCG and 2 at AMC and LUMC. After spatial realignment to the first image, a mean EPI was created. The movement parameters for each participant were inspected. If a participant moved more than 3 mm in any direction (anterior-posterior, right-left, inferior-superior) the data were excluded from further analysis. The anatomy scan was coregistered to the mean EPI image. Subsequently, T1, and with it EPI images, were spatially normalized to a standard stereotaxic space (Montreal Neurological Institute). During normalization, data were resampled into a 3 x 3 x 3 mm grid with 7th degree B-spline interpolation. The functional data were smoothed with a 3D isotropic Gaussian kernel of 8 mm full-width at half-maximum.

Imaging analysis

Low-frequency noise was removed by applying a high-pass filter (cut-off of 128 s) to the fMRI time-series at each voxel. Significant hemodynamic changes for each condition were calculated using the general linear model (Friston et al., 2004) with respect to the event-related response convolved with canonical hemodynamic response function. To identify activity in regions related to face processing, we computed a t-contrast of all faces combined to the baseline, and tested this contrast at $p < 0.05$ family wise error (FWE) corrected for multiple comparisons. To the test the hypotheses between the relation of neuroticism and negative affect, the analyses were subsequently restricted to the negative emotional facial expressions. T-contrasts for "angry > neutral", "fearful > neutral", "sad > neutral" were calculated for each subject. Results of these weighted contrast (contrast images) were then entered in a second level random effect model. For each negative emotional facial expression (compared to neutral), neuroticism, extraversion, age and gender were entered as regressors. A one-sample t-test was applied to test the positive and negative effect of the neuroticism scores regressor. Effectively, this analysis corresponds to detecting semi-partial correlations between brain activity and neuroticism, when correcting for extraversion, age and gender. Since the amygdala, the ACC and the DMPFC all shown to have neuroticism dependent variation in activity when processing emotional stimuli, they were defined as regions of interest (ROI). The amygdala and ACC volumes were based on the WFU pickatlas (Maldjian et al., 2003). The DMPFC was defined as a 10mm sphere around the peak voxel coordinates reported in a study on phobic pronenesss in relation to the processing of negative emotional faces (Rubino et al., 2007). We applied an initial significance threshold of $p<0.005$ (uncorrected) and a spatial extent of five voxels ($k \geq 5$), restricted to our a priori regions of interested (ROI): the amygdala, the ACC and the DMPFC. Furthermore we report activation outside our ROIs at $p<.001$, $k \geq 10$ voxels uncorrected for multiple comparisons. Activations are reported in standard Montreal Neurological Institute (MNI) space.

Functional connectivity analysis: psycho-physiological Interaction (PPI)

Psycho-physiological Interaction (PPI) analyses were used to assess how activity in a brain region of interest covaries with a source region in response to the experimental condition (Friston et al., 1997). Within each condition (negative emotional faces compared to neutral faces), we separately examined functional connectivity from the left and right amygdala as a source region. To identify the amygdala activation for

each participant we examined the contrast of all faces compared to the baseline at p<.05 uncorrected. The deconvolved time series from a 5 mm radius sphere around the individually defined peak activated voxel within the amygdala (defined by the WFU pickatlas mask) (Maldjian et al., 2003) was extracted (44 participants). The PPI was calculated as the element by element product of the left and the right amygdala time series (the first eigenvariate from all voxels' time serie) and a vector coding for the effect of task ("anger > neutral", "fear > neutral", and "sad > neutral"). This product was subsequently re-convolved with the hemodynamic response function (HRF). This interaction term was then entered as a regressor in a first level model together with the time series of the amygdala and the vector coding for the task effect. The models were estimated and contrasts generated to test the effects of positive and negative PPIs. This analysis identified regions that display stronger functional connectivity with the amygdala for an emotional compared to a neutral facial expression, and neutral compared to emotional respectively.

The contrast images for the PPI effects were then entered in a second level analysis. In a similar manner as the conventional analysis, neuroticism, extraversion, age and gender were entered as regressors. Subsequently, the positive and negative effect of neuroticisms were tested, which identified brain regions that showed connectivity with the amygdala correlating positively or negatively with neuroticism scores, respectively. We applied an initial uncorrected threshold of p<.005, k ≥ 5, restricted to our a priori regions of interest, the ACC, the DMPFC, DLPFC and VMPFC, and report family wise error (FWE) small volume corrections (SVC) of p<.05 where applicable. The ACC mask was based on the WFU pickatlas, while the DMPFC, DLPFC and VMPFC (lateral orbitofrontal cortex) masks were defined as a 15 mm sphere around the peak coordinates reported in a study on amygdala connectivity based on a large fMRI data set on processing angry and fearful faces (Stein et al., 2007a). Furthermore we report activation outside our ROIS at p<.001, k ≥ 10 voxels uncorrected for multiple comparisons.

2.4 Results

Behavioral results

For the entire group, reaction times for the different emotional faces were: angry mean RT = 825 ms, SD = 158, fear RT = 879, SD = 166, sad RT = 874, SD = 163 and neutral RT = 888 SD = 155. There was a main effect of emotion on reaction time, driven by a faster RT for angry compared to neutral faces $t(55)$=-7.6, p<0.05. Accuracy overall was high: for angry 98.3%, fear 98.5%, sad 96.1% and neutral 95.4%. There were no significant correlations between neuroticism (or extraversion) and the differences scores of each negative compared to neutral facial expression or for accuracy, for each correlation p>0.05.

Personality scores

The sample scores for neuroticism were mean 24.3 (range: 13-36), SD = 5.3. For extraversion these scores were mean 44.4 (range: 27-56), SD = 6.6. There was a significant negative correlation between neuroticism and extraversion, r = -0.49, p < 0.05. Since individual differences in extraversion also influence emotion processing, we aimed to exclude any possible effect by adding extraversion as a regressor in our model (see Passamonti et al., 2008).

fMRI results

Main effects of emotional faces versus baseline

We compared all emotional faces together against the scrambled faces baseline to assess activity related to face processing. Main effect of the face processing were found in the bilateral fusiform gyrus (left; -42/-54 /-24, Z=6.1 k=25; right, 39 / -45 /-24, Z=7.26, k=110), bilateral amygdala (left; -18 / -6 / -15, Z=6.95, k=126, ; right 21 /-6 /-15, z-=7.13, k=82), and the right inferior frontal gyrus (51/27/21, Z=6.37, k=174). All activations were p<.05, whole brain FWE corrected.

Brain activity for emotional versus neutral faces and relation with neuroticism

In order to identify activity in brain regions that varied as a function of neuroticism scores, we tested the effect of neuroticism in a regression model. This resulted in the identification of activity in brain regions that was positively or negatively correlated with neuroticism in response to emotional facial expressions compared to neutral facial expressions. Table 1.1 shows activations clusters and peak coordinates. As one can see, of our a priori regions of interest, only the right DMPFC showed an effect of neuroticism. We found a positive relation between activity in this region for fearful compared to neutral emotional faces and neuroticism scores. The main effects of each contrast (regardless of individual differences) are presented in table S2.1

CHAPTER 2

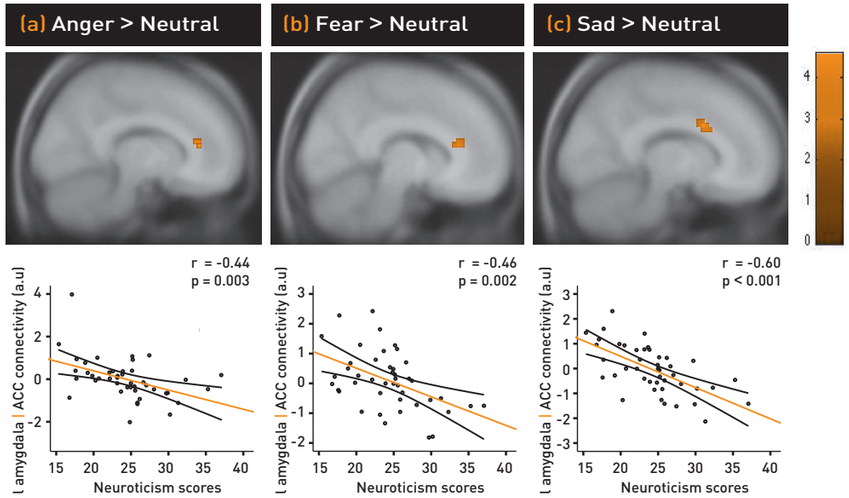

Figure 2.1

Brain regions displaying association between neuroticism and functional connectivity with the left amygdala for emotional compared to neutral faces. (a) right anterior cingulate cortex for angry compared to neutral facial expressions. (b) right anterior cingulate cortex for fearful compared to neutral facial expressions (c) right dorsal anterior cingulate cortex for sad compared to neutral facial expressions.

Functional connectivity : PPI analysis

In order to investigate how neuroticism is associated with functional connectivity of the amygdala and the prefrontal cortex, we tested the effect of neuroticism as a regressor in a model of connectivity with the left and right amygdala (separately) as source regions. This analysis resulted in the identification of brain regions showing connectivity with the amygdala that was either positively or negatively correlated with neuroticism scores when viewing emotional compared to neutral facial expressions.

Table 2.2 shows the modulatory effect of neuroticism on the connectivity of the left amygdala. For angry and fearful faces, connectivity of the left amygdala and right ACC was negatively associated with neuroticism scores. For sad compared to neutral faces, a similar relation between

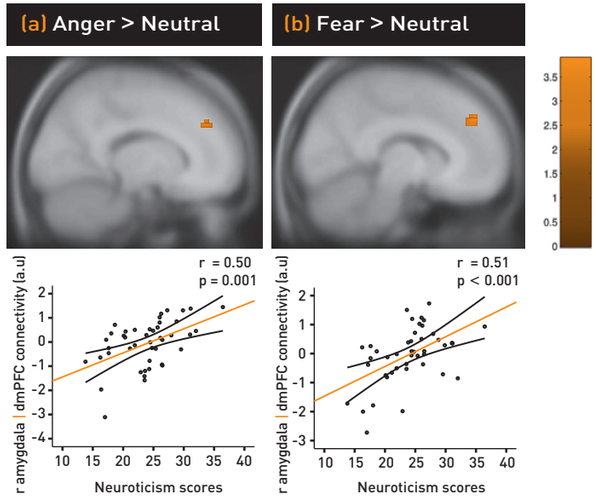

Figure 2.2

Brain regions displaying association between neuroticism and functional connectivity with the right amygdala for emotional compared to neutral faces. (a) right dorsomedial prefrontal cortex for angry compared to neutral facial expressions. (b) right dorsomedial prefrontal cortex for fearful compared to neutral facial expressions.

neuroticism and amygdala connectivity (with a more dorsal part of the ACC) was observed. Both these findings indicate that the higher the neuroticism scores, the lower the functional coupling for negative emotional compared to neutral facial expressions between the left amygdala and the ACC. These effects are shown in Figure 2.1. For display purposes the partial correlation scores for neuroticism (the residual, corrected for extraversion, age and gender) were linear transformed (mean added and and scaled standard deviation) to approximate the original neuroticism scores.

Table 2.3 displays the positive and negative correlations between neuroticism and regions functionally coupled with the right amygdala for each of the negative emotional compared to neutral emotional facial expressions. A positive correlation was found between the amygdala connectivity with the right dorsomedial prefrontal (DMPFC) cortex for both angry and fearful compared to neutral facial expressions. This indicates that the higher the neuroticism scores, the

Contrast	Region	Side	Voxels	Z values	P values	MNI coordinates x	y	z
angry > neutral								
fear > neutral								
Positive	DMPFC	**R**	**10**	**2.95**	**0.002**	**6**	**57**	**33**
	Calcarine gyrus	L	17	3.71	<0.001	-3	-93	6
sad > neutral								
Positive	Posterior Cingulate gyrus	R	10	3.65	<0.001	6	-48	30

Table 2.1 Brain areas displaying a correlation between neuroticism scores and activity for angry, fearful and sad facial expressions.
Note: A priori regions of interest are shown in bold. DMPFC: dorsomedial prefrontal cortex. Other activations at a threshold of $p<0.001$, and minimal 10 contiguous voxels are also reported. No negative correlations were found.

Contrast	Region	Side	Voxels	Z values	P values	MNI coordinates x	y	z
angry > neutral								
Positve	Parahypocampal gyrus	R	12	4.39	<0.001	30	-39	-6
Negative	ACC	**R**	**7**	**2.96**	**0.002**	**12**	**36**	**12**
fear > neutral								
Negative	ACC	**R**	**15**	**3.26**	**0.001**	**9**	**30**	**15**
sad > neutral								
Negative	**Dorsal ACC***	**R**	**41**	**4.08**	**<0.001**	**12**	**12**	**30**

Table 2.2 Association between neuroticism and functional connectivity with the left amygdala for angry, fearful and sad facial expressions.
Note: A priori regions of interest are shown in bold. *Small volume corrected, FWE $p<.05$ ACC; Anterior Cingulate Cortex. Other activations at a threshold of $p<.001$, and minimal 10 contiguous voxels are also reported.

Contrast	Region	Side	Voxels	Z values	P values	MNI coordinates x	y	z
angry > neutral								
positive	DMPFC	**R**	**16**	**3.37**	**0.001**	**21**	**42**	**36**
fear > neutral								
Positive	DMPFC*	**R**	**49**	**3.22**	**0.001**	**21**	**42**	**36**
	DMPFC*	**L**	**15**	**3.26**	**0.001**	**-6**	**48**	**36**
sad > neutral								
Positive	IFG	L	23	3.69	<0.001	-27	42	3

Table 2.3 Association between neuroticism and functional connectivity with the right amygdala for angry, fearful and sad facial expressions.
Note: A priori regions of interest are shown in bold. *Small volume corrected, FWE $p<.05$ DMPFC: dorsomedial prefrontal cortex; IFG inferior frontal gyrus. Other activation at a threshold of $p < 0.001$, and minimal 10 contiguous voxels are also show

Contrast	Region	Side	Voxels	Z values	P values	MNI coordinates x	y	z
angry > neutral								
	MTG	R	19	3.85	<.001	54	-42	6
	MTG	L	24	3.76	<.001	-54	-60	0
fear > neutral								
	MTG	R	10	3.8	<.001	51	-39	6
	MTG	L	14	3.75	<.001	-57	-60	0
sad > neutral								
	Cerebellum	L	125	4.81	<.001	-18	-57	-21
	Post central gyrus	R	81	4.6	<.001	48	-24	54
	MTG	R	25	3.73	<.001	57	-34	0
	IFG	R	15	3.66	<.001	54	30	3
	Posterior cingulate gyrus	R	10	3.54	<.001	60	-36	30

Table S2.1 Main effects of each negative emotional compared to neutral facial expressions
Note: MTG: medial temporal gyrus, IFG: inferior frontal gyrus

stronger the coupling for fearful and angry compared to neutral faces between the right amygdala and the right DMPFC (see Figure 2.2).

It is important to note that within each of the neuroticism related connectivity effects in the ACC and DMPFC there were no significant main effects (irrespective of individual differences) of each contrast, even at a threshold of $p<.05$ uncorrected. Within our other regions of interest we only found significant effects of a positive PPI for the fear > neutral contrast in ventral lateral prefrontal cortex (-36/33/-8, Z=3.58), $p<.05$ FWE, small volume corrected. No other contrasts showed a positive or negative PPI effect in our ROIS

2.5 Discussion

Neuroticism modulates brain activity in the DMPFC during the processing of fearful faces

In this study, we investigated the modulatory role of neuroticism on brain activity and functional connectivity while processing negative compared to neutral facial expressions. Our results showed that activation in the DMPFC varied as a function of neuroticism scores in response to fearful facial expressions. This finding is broadly in line with studies in which DMPFC activity was found to be related to neuroticism (Haas, Constable, & Canli, 2008) and phobic proneness (Rubino et al., 2007) during the processing of sad, respectively fearful and angry facial expressions. Both studies discuss these findings within a framework of self-referential processing, a construct strongly associated with this brain region (Amodio & Frith, 2006; Northoff & Bermpohl, 2004). In accordance with these notions, neuroticism has been associated with an individual's self-schema, *i.e.* a '[…] constellation of self-referent information of one's own unique traits […]' which '[...] serves to guide the processing of personally relevant information' (Winter & Kuiper, 1997). Trapnell and Campbell (1999) found that neuroticism related positively to *ruminative* self-consciousness (but not to *reflective* self-consciousness), which is associated with psychological distress (Trapnell & Campbell, 1999). Hence, our results may imply that higher levels of neuroticism are associated with a higher degree of self-referential negative appraisal during the processing of fearful expressions

Neuroticism modulates amygdala–ACC and amygdala–DMPFC connectivity

We did not observe an effect of neuroticism on activity in the amygdala during processing of negative emotional expressions. However, and crucial to our hypothesis, connectivity analysis showed that neuroticism distinctively modulated connectivity between the 'left amygdala – right ACC' and 'right amygdala – right DMPFC'. We found no significant main effect for these regions, which is broadly in line with previous research, applying the same functional connectivity measure, that showed relatively small effects of functional coupling between the amygdala and these prefrontal regions when processing fearful compared to neutral faces (Williams, 2006). Our results suggest that it is worthwhile to account for individual differences in neuroticism when studying functional connectivity related to the processing of negative emotional facial expressions.

We found that connectivity of the left amygdala with the ACC for angry and fearful, and dorsal ACC for sad facial expressions, correlated negatively with neuroticism scores. This indicates that subjects high in neuroticism, while processing negative compared to neutral emotional expression, displayed relatively less amygdala and ACC functional coupling. Numerous studies have shown the importance of amygdala–ACC functional connectivity in the context of emotion processing. For example, Etkin, Egner, Peraza, Kandel, & Hirsch (2006), found that during high conflict trials in an emotional Stroop paradigm the amygdala and the rostral part of the ACC were negatively functionally coupled, suggesting an inhibitory role of the ACC over the amygdala. In line with our findings, a study on trait anxiety (Kienast et al., 2008) showed a negative correlation of this trait with amygdala-ACC connectivity when viewing negative compared to neutral scenes. Moreover, Pezawas et al. (2005) found that carriers of the short allele of the serotonin transporter gene (a polymorphism related to anxiety) showed relatively less functional coupling between the amygdala and ACC when processing angry and fearful faces. These findings suggest that persons high in neuroticism (or other individual differences related to anxiety) display less ACC related inhibitory control over the amygdala. It is of interest that the opposite pattern was found for a personality trait associated with positive affect and approach motivation; the behavioral activation system (BAS) (Passamonti et al., 2008). These authors found that BAS positively predicted amygdala-ACC connectivity for angry compared to neutral faces. The studies mentioned above demonstrate the relevance of amygdala–ACC connectivity in emotion processing and emotion regulation. These reports suggest that individual differences in personality traits

modulate amygdala-ACC functional connectivity, and that the direction of this correlation is different for traits related to negative or positive affect.

In contrast to our results regarding left amygdala–ACC connectivity, we found a positive relation between neuroticism scores and right amygdala - right DMPFC connectivity. Participants with higher scores on neuroticism displayed relatively enhanced connectivity between the right amygdala and right DMPFC during the processing of angry and fearful compared to neutral faces. This finding relates to a study on functional connectivity in generalized social phobia (GSP) while processing self-referential praise and criticism (Blair et al., 2008). Their results showed that patients (compared to healthy control participants) displayed stronger functional connectivity between amygdala and that the DMPFC for self-referential criticism. The authors argue that this finding may '[…] reflect a negative attitude toward the self, particularly in response to social stimuli [...]', and MPFC may modulate amygdala engagement to initiate and maintain aspects of GSP (Blair et al., 2008). In keeping with our finding of neuroticism related differences in DMPFC *activity*, our amygdala – DMPFC *connectivity* results also suggest that persons high in neuroticism might demonstrate stronger self-referential processing in response to negative emotional faces.

There is substantial evidence for the role of neuroticism in the development of, for example, anxiety disorders (Bienvenu et al., 2001; 2004). The pattern we found in the relation between functional connectivity and neuroticism might provide insight in the neural basis of neuroticism-linked susceptibility to negative affect, and its associated vulnerability for the development of affective disorders. Taken together, our amygdala-ACC connectivity results indicate that high levels of neuroticism are associated with relatively less inhibitory control over negative facial expressions. Based on DMPFC activity and amygdala – DMPFC connectivity, we suggest that those high in neuroticism demonstrate stronger self-reference to negative facial expressions. Furthermore, it is of interest to note the apparent dissociation between neuroticism and connectivity from the left and the right amygdala. A recent meta-analysis on amygdala function showed evidence for a dissociation between the left and right amygdala regarding temporal dynamics (Sergerie et al., 2008), but did not find evidence for specific interactions between amygdala lateralization and valence or gender (Sergerie et al., 2008) in line with a previous meta-analysis (Baas et al., 2004). Less is known regarding lateralization of amygdala connectivity, and future research should therefore further explore possible lateralization in functional pathways from the amygdala.

Limitations

The interpretation of our connectivity analysis is restricted by the inherent limitations of functional connectivity measures. In our application of psycho-physiological interaction analysis, it is a measure of *functional,* but not *effective* connectivity (Friston et al., 1997). The main difference between these concepts is that the former is a correlation method and its results do not imply a causal relation between regions involved. PPI analysis in and of itself is therefore insufficient to assess the direction of effects (i.e., reciprocal or unidirectional) between the amygdala and the DMPFC and ACC. This is an important limitation considering, for example, the argued regulatory role of the ACC over the amygdala. Nonetheless, other studies, applying different methodologies, have provided more direct evidence for a top-down regulatory role of the ACC over the amygdala. One tracing study, for example, showed that the ACC has more projections to the amygdala than vice versa (Ghashghaei, Hilgetag, & Barbas, 2007). Furthermore, deep brain stimulation experiments in depressed patients suggest that stimulating the ACC -through its connectivity pathways- affects several subcortical regions, including the amygdala (Johansen-Berg et al., 2008; Mayberg, Lozano, Voon, & McNeely, 2005).

In this experiment, we found differences in amygdala – prefrontal connectivity associated with neuroticism during an emotion processing task, with only very mild demands on cognitive control over emotional functions. It is therefore very well possible that when engaged in more cognitively demanding tasks, additional amygdala – PFC connectivity pathways strongly involved in inhibitory control, would show associations related to individual differences in personality. For example, some studies have shown subcortical – prefrontal connectivity in relation to functions such as reappraisal (Banks et al., 2007; Wager et al., 2008), extinction learning (Quirk and Beer, 2006) and response conflict (Etkin et al., 2006). Future research on negative affect should therefore incorporate individual differences in neuroticism in functional connectivity on such more challenging emotion regulation tasks. We would argue that in such paradigms individual differences are likely to be associated with, for example, amygdala – ventromedial or ventral lateral prefrontal connectivity.

Conclusion

Our present study indicates that individual differences in neuroticism are of importance in modulating functional connectivity of amygdala and prefrontal regions when processing negative emo-

tional material. Neuroticism was negatively associated with amygdala-ACC, and positively related to amygdala – DMPFC connectivity, when processing negative emotional facial expressions. These findings may provide insight into the neural mechanisms associated with susceptibility to negative emotional material, and may be relevant for to the development of affective disorders.

Chapter 3

Extraversion is linked to volume of the orbitofrontal cortex and amygdala

Henk Cremers | Marie-José van Tol | Karin Roelofs | André Aleman
Frans G. Zitman | Marc A. van Buchem | Dick J. Veltman | Nic J.A van der Wee

PLOS ONE, 2011, 6(12), E28421

3.1 Abstract

Objective: Neuroticism and extraversion are personality factors associated with the vulnerability for developing depression and anxiety disorders, and are possibly differentially related to brain structures implicated in the processing of emotional information and the generation of mood states. To date, studies on brain morphology mainly focused on neuroticism, a dimension primarily related to negative affect, yielding conflicting findings concerning the association with personality, partially due to methodological issues and variable population samples under study. In the present study neuroticism but also, extraversion, a dimension primarily related to positive affect, are investigated in relation to brain volume using an optimized Voxel Based Morphometry (VBM) approach.

Methods: High resolution structural T1-weighted MR images of 65 healthy adults were processed using an optimized VBM approach. Multiple regression analyses were performed to test for associations of neuroticism and extraversion with prefrontal and subcortical volumes.

Results: Orbitofrontal and right amygdala volume were both positively related to extraversion. Extraversion was differentially related to volume of the anterior cingulate cortex in males (positive) and females (negative). Neuroticism scores did not significantly correlate with these brain regions.

Conclusions: As extraversion is regarded a protective factor for developing anxiety disorders and depression, and has been related to the generation of positive affect, the present results indicate that the reduced likelihood of developing affective disorders in individuals high on extraversion is related to modulation of emotion processing through the orbitofrontal cortex and the amygdala.

3.2 Introduction

Neuroticism and extraversion are personality factors that have been directly linked to emotional states: neuroticism has been associated with susceptibility to negative emotional states, whereas extraversion has been linked to susceptibility to positive emotional states (Larsen & Ketelaar, 1991). Also, neuroticism correlates positively, whereas extraversion correlates negatively, with (subsyndromal) symptoms of depression and anxiety in the general population (Jylhä & Isometsä, 2006). Other personality traits, such as agreeableness, openness to experiences, and conscientiousness, have been proposed to play a more indirect role in influencing affective states (Larsen & Ketelaar, 1991). Consequently, neuroticism and extraversion may respectively put one at risk for (Bienvenu et al., 2004; Clark, Watson, & Mineka, 1994) or protect one against (Clark et al., 1994) development of affective disorders such as depression, panic disorder and social anxiety disorder.

More insight into the relation between personality and brain regions associated with emotion processing and regulation may help to illuminate how personality is involved in the processing of emotional information, and hence, in vulnerability to mood and anxiety disorders. On a functional level, it has previously been shown that neuroticism modulates neural activity in prefrontal and subcortical brain regions related to affective processing (Canli, 2004; Cremers et al., 2010). Neuroticism has been related to more amygdala activation in response to distracting negative facial expressions during a cognitive task (Haas, Omura, Constable, & Canli, 2007), and to less anterior cingulate cortex (ACC)–amygdala connectivity during processing of negative emotional facial expressions (Cremers et al., 2010). Extraversion on the other hand, was found to be positively associated with amygdala activation in response to happy faces (Canli, Sivers, Whitfield, Gotlib, & Gabrieli, 2002). Together, these findings indicate that core brain structures involved in emotion processing (e.g. the amygdala, the anterior cingulate gyrus, and the ventral part of the prefrontal cortex including the orbitofrontal cortex –OFC-) may play a role in the relation between personality, the processing of emotional information, and the production of mood states (Mobbs, Hagan, Azim, Menon, & Reiss, 2005).

Structural variation might underlie the relation between personality and activity in brain regions related to emotion perception and appraisal. In an adolescent sample, a positive association between extraversion and (medial) prefrontal volume (Blankstein, Chen, Mincic, McGrath, & Davis, 2009) has been observed, which has also been demonstrated in adults (DeYoung et al., 2010), although not consistently (Wright et al., 2006). Therefore, the relation between brain vol-

ume and affect-related personality traits remains unclear. Inconsistencies in reported findings may be due to methodological and technical issues, such as the use of different segmentation and normalization strategies (e.g. analyses based on modulated vs unmodulated images (Omura, Todd Constable, & Canli, 2005), not accounting for total brain volume (Jackson, Balota, & Head, 2009), or to sample characteristics and processes of brain maturation). For example, Jackson et al., 2009, and Wright, Feczko, Dickerson, & Williams (2007), studied the relation between neuroticism and regional brain volume in an elderly population, whereas Blankstein et al., 2009 included an adolescent sample. As aging is an important predictor of regional brain volume, and because the process of aging (including brain maturation) has been shown to interact with personality (Jackson et al., 2009), it is possible that the conflicting finding may depend on the age range of the sample. It is therefore necessary to further elucidate the complex relation between affect-related personality traits and regional brain volume in an adult sample, controlling for these important factors (i.e. age, total brain volume and modulation) but also testing for possible interaction effect with sex, as Blankstein et al., (2009) suggested that personality differentially affects regional brain volume in male and female adolescents.

In the present study, we used an optimized VBM approach to investigate the relationship between neuroticism, extraversion, and regional brain volume in a large sample of healthy adults. We focused on brain regions involved in the initial processing of emotional information (amygdala) and on regions related to the appraisal and decision making influence of emotional information, ACC and orbitofrontal cortex (Wright et al., 2006). As such, we aimed to identify neuro-anatomical substrates associated with affect-related personality traits.

3.3 Methods

Ethics Statement

The study was carried out in accordance with the Declaration of Helsinki. Also, the Ethical Review Boards of the Leiden University Medical Center (LUMC), Academic Medical Center (AMC), University of Amsterdam, and University Medical Center Groningen (UMCG) approved this study. All participants provided written informed consent after complete description of the study.

Participants

Sixty-five healthy participants were selected from the NESDA (Netherlands Study of Depression and Anxiety) neuroimaging study (Penninx et al., 2008). The MRI main sample is described in detail elsewhere (van Tol et al., 2010). Exclusion criteria for the current sample were: 1) a history of or current DSM IV axis I pathology, 2) taking any psychoactive drugs, 3) the presence or history of major internal or neurological disorder, 4) dependency or recent abuse (past year) of alcohol and/or drugs, 5) hypertension 6) general MR-contraindications.

Personality questionnaire

To asses personality traits, all participants completed the NEO Five Factor Inventory (Costa & McCrea, 1992).

Image acquisition

Imaging data were acquired using Philips 3T MR-systems (Best, The Netherlands) located at the Leiden University Medical Center (LUMC), Academic Medical Center (AMC), University of Amsterdam, and University Medical Center Groningen (UMCG), equipped with SENSE-8 (LUMC and UMCG) and SENSE-6 (AMC) channel head coils, respectively. For each subject, anatomical images were obtained using a sagittal 3D gradient-echo T1-weighted sequence (TR=9 ms, TE=3.5 ms; matrix 256x256; voxel size: 1 x 1 x 1 mm; 170 slices).

Data analysis

VBM following the Diffeomorphic Anatomical Registration Through Exponentiated Lie (DARTEL) algebra software (Ashburner, 2007) implemented in Matlab 7.1.0 (The Matlab Inc, Natick, MA, http://www.mathworks.com/) was used. The preprocessing and masking procedure is described in detail elsewhere (van Tol et al., 2010). Briefly, after segmentation, data were registered, normalized, and modulated using the DARTEL pipeline. Grey matter (GM) images were normalized to Montreal Neurological Institute (MNI) space and smoothed at 8 mm full width at half maximum (FWHM). In the resulting images, each voxel represents an absolute amount of brain volume, equivalent to the brain volume per unit prior to normalization.

Next, we performed a multiple regression analysis with neuroticism and extraversion as independent variables and voxel-wise GM density maps as the dependent factor. In addition, sex x personality interaction terms were calculated by setting up a flexible factorial design. In each model, age, scan center, and GM total volumes were entered as nuisance variables. Based on the literature on emotion processing in affective disorders (Drevets, Price, & Furey, 2008), we set the following regions of interest (ROI): amygdala, orbitofrontal cortex (OFC), anterior cingulate cortex (ACC). A family wise error (FWE) at $p<.05$ correction for the spatial extent of the ROIS (small volume corrections) was applied. The ROIS were defined by the Automated Anatomical Labeling (AAL) templates implemented in the Wake Forest University School of Medicine (WFU) pickatlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). For regions other than the ROIS, a voxel level threshold of $p<.05$ whole brain FWE corrected was set *a priori*. For completeness, explorative analysis are performed at an uncorrected threshold of p<.001, with a spatial cluster extent of 25 contiguous voxels. Demographic and clinical data were analyzed with SPSS 16.0 (http://www-01.ibm.com/software/analytics/spss/) and significance was set at $p<.05$.

3.4 Results

Sample characteristics and personality scores

The age range of the 65 participants (42 females) was: 21-56, Mean (M)=40.5, standard deviation (sd)=9.7; years of education: M=14.3, sd=2.9. Neuroticism (range=13-36; M=24.2, sd=4.9) correlated negatively to extraversion (range 27-56; M=43.7, sd=6.2; r=-0.38, $p=.002$). Neuroticism correlated negatively with total GM, whereas Extraversion correlated positively with total GM (Neuroticism: r=0.29, $p=.02$; Extraversion: r=.29, $p=.02$). After partialling out variations in age and sex, only the correlation between Extraversion and GM total remained significant (Neuroticism: $r_{partial}=-.17$, $p=.18$; Extraversion: $r_{partial}=.27$, $p=.03$). Neither age nor years of education were significantly correlated with either neuroticism or extraversion (all $p>.13$), and no effect of sex was observed ($F<1.74$, $p>.19$).

Personality and regional brain volume

Volumes of both the right medial OFC -BA 11- (encompassing the rectal gyrus, the orbitofrontal region of Brodmann area 13 and the subgenual cingulate gyrus -BA 25-) and the right centro-medial

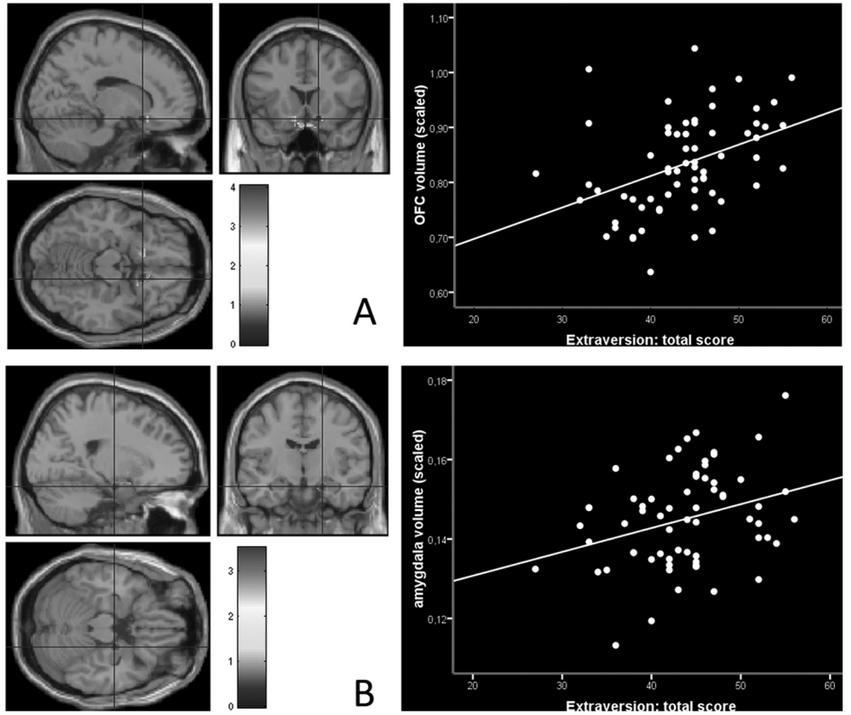

Figure 3.1 Correlations of Extraversion and volume in the OFC and amygdala
A) Positive correlation of extraversion and volume of the right orbital frontal gyrus scaled for total gray matter volume (r=.33; $r_{partial}=.47$ when neuroticism and age were partialled out) . B) Positive correlation of extraversion and right amygdala volume scaled for total gray matter volume (r=.31, $r_{partial}=.39$ when neuroticism and age were partialled out). Mean volume in ml of the significant voxels was extracted per subject and divided by total GM volume. In the correlation plots, mean volume of the significant amygdala and ofc region is depicted in scaled volume in ml * 10^{-3}.

amygdala were positively related with extraversion at the set threshold ($p_{FWE}<.05$ corrected for extent of ROI). At $p<.001$ uncorrected, the positive correlation of extraversion was also observed in the left OFC. Neuroticism did not show a significant relation with regional brain volume in any of

POSITIVE CORRELATIONS OF EXTRAVERSION				MNI-coordinate				
R/L	region	BA	k	x	y	z	Z-score	r
OFC								
R	medial OFC, subgenual cingulate gyrus	11/25	329	13	16	-14	3.77	.47
	medial OFC, rectal gyrus	11		2	22	-18	3.54	.45
L	medial OFC, subcallosal gyrus	34	68	-20	12	-14	3.33	.42
amygdala								
R	amygdala, dorsal subdivision	n.a.	31	21	-10	-21	3.34	.42
NON-ROI EFFECTS								
R	superior parietal lobule	7	60	34	-65	58	4.18	
L	cerebellar declive, posterior lobe	n.a.	65	-56	-65	-29	3.93	
L	posterior cingulate gyrus	31	63	-7	-41	27	3.60	
NEGATIVE CORRELATIONS OF EXTRAVERSION				MNI-coordinate				
R/L	region	BA	k	x	y	z	Z-score	
R	precentral gyrus	4	144	41	-20	47	3.75	
				34	-16	46	3.65	
L	calcarine sulcus		30	-16	-69	13	3.55	
L	inferior frontal gyrus	45	59	-49	20	14	3.46	
L	superior parietal lobule	7	65	-21	-54	61	3.45	
POSITIVE CORRELATIONS OF NEUROTICISM				MNI-coordinate				
R/L	region	BA	k	x	y	z	Z-score	
L	middle temporal gyrus	21	278	-64	-43	-2	3.86	
NEGATIVE CORRELATIONS OF NEUROTICISM				MNI-coordinate				
R/L	region	BA	k	x	y	z	Z-score	
R	supplemental motor area (SMA)	6	45	13	-20	68	3.57	

Table 3.1 VBM effects
R/L: Right vs. Left hemisphere; BA: Brodmann area; k=clustersize at *p<.001*, uncorrected; r=correlation coefficient at peak voxel.

the ROIs. Adding the other three personality variables (openness, agreeableness, and conscientiousness) from the NEO FFI did not significantly change the results (OFC: Z=3.28; amygdala, Z=3.50). Results are shown in figure 3.1 and table 3.1. No significant whole brain FWE $p<.05$ corrected effects outside these ROIs were found. For completeness, positive and negative correlations of neuroticism and extraversion that were observed at $p<.001$, uncorrected with a spatial cluster extent of 25 contiguous voxels are listed in table 3.1 (*non-ROI effects*). However, these effects will not be further discussed, as these were not part of our *a priori* set regions of interest.

Extraversion x sex interaction analysis showed an effect in the pregenual ACC (MNI coordinate: [x=3, y=48, Z=7] (p=.01 FWE corrected for the volume of the bilateral ACC as defined by the AAL templates). Post-hoc analysis showed that males displayed a significant positive association with extraversion (β=.40, p=.008, 95% C.I.(B)=[0.005 – 0. 26], zero-order correlation: r=.43), with GM totals, neuroticism, age, and scan center added to the model, whereas the association was in the opposite direction in female (β=-.28, p=.006, 95% C.I.(B)=[-0.24 – -0.004], zero-order correlation: r=.15). Sex by neuroticism interactions were not observed in any other region of interest.

3.5 Discussion

In the present report, we used an optimized VBM approach to test for personality related variations in regional brain volume in an adult sample. While controlling for age, sex and total GM we demonstrated positive correlations between extraversion and regional brain volume in the medial OFC and centro-medial amygdala. This result confirms the role of the OFC in personality, a region that was already associated with personality changes in the case report of Phineas Gage (Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994). We also found a positive correlation between extraversion and total gray matter volume. However, we did not find strong structural correlates of neuroticism.

The observation that extraversion correlated positively with volume of both the medial OFC (extending into subgenual ACC area 25) and the amygdala is of interest because of the role these regions play in affective processing (Rolls, 2004; Rolls & Grabenhorst, 2008). The medial OFC has often been associated with controlling reward and punishment related behavior, emotion regulation, approach related behavior (Milad & Rauch, 2007) and decision making (Kringelbach & Rolls, 2004; Price, 2003), and has projections to visceral control structures, such as the ventral striatum, amygdala, hypothalamus, periaqueductal grey, and hippocampus: regions that are criti-

cal in modulating behavior and emotional expression (Milad & Rauch, 2007; Price, 2003; Price & Drevets, 2010). The amygdala has a well-documented role in emotion processing and has bi-directional connections with the medial OFC (Price, 2003). Interestingly, a positive correlation of OFC thickness with extraversion and fear extinction has been previously described (Rauch et al., 2005). In another study, humor-driven activation, reflecting hedonic capacity, was found to be positively correlated with extraversion in the right medial OFC (Mobbs et al., 2005). Moreover, in the amygdala, activation during happy face viewing was found to be positively related to extraversion (Canli, 2004). Hence, the increased volumes of medial OFC and amygdala may play a role in the increased sensitivity to positive, pleasant information and (social) reward, and thus, the propensity to experience the positive affect which characterizes extraversion (Clark et al., 1994). The nature of this role, however, awaits further elucidation, as relations between volume and function are not straightforward: increased volumes of GM in brain areas may be reflective of a number of processes involving, among others, glial cells, inhibitory or excitatory neurons and interneurons. However, these processes cannot as yet be assessed in vivo in humans.

Beside the overall positive correlation between OFC and amygdala volume and extraversion, we also found a sex x extraversion interaction in the ACC. Males showed a positive correlation between ACC volume and extraversion, while this correlation was negative in females. A similar effect in the medial prefrontal gyrus was previously shown in adolescents (Blankstein et al., 2009). These findings could suggest that, in men, the ACC is included in the same extraversion mediated regulatory network as the amygdala and OFC, while this is not the case in females. This could imply that extraversion has a stronger protective effect in men than in women, in line with the well known observation that men are less susceptible to affective disorders (Piccinelli & Wilkinson, 2000).

It is also interesting to note that our findings were mainly right-lateralized. In a study by Hastings, Parsey, Oquendo, Arango, & Mann (2004), it was found that non-medicated depressed patients showed smaller right amygdala volume than controls (an effect which was driven by the female participants). Since we found that low extraversion is linked to smaller right amygdala volume, our results can be considered in line with this study. Moreover, a meta-analysis on lateralization has found that the right amygdala is more involved in processing masked stimuli, whereas the left amygdala is more involved in processing stimuli which contain language (Costafreda, Brammer, David, & Fu, 2008). Relating this finding to our data, it might suggest that extraversion is more strongly linked to the subconscious emotion-processing role of the amygdala. However,

the lack of consensus regarding lateralization and amygdala function among functional MRI meta-analyses should be noted (Baas, Aleman, & Kahn, 2004; Sergerie, Chochol, & Armony, 2008; Wager, Phan, Liberzon, & Taylor, 2003). Moreover a positive correlation between the left amygdala and extraversion was also observed at a more liberal threshold ($p<.005$ uncorrected), and no formal interaction of extraversion x lateralization was observed in a repeated measures ANOVA. Therefore, no strong statements on lateralization can be made. Furthermore the positive correlation between extraversion and right amygdala volume was localized in the central-medial amygdala, which contains (most of) the efferent connections from the amygdala to the brainstem and hypothalamus (Price, 2003). These connections are particularly important for fight/flight responses, and taken together, our findings might fit with the idea that extraversion is linked to moderating unconscious emotion processing and primary stress responses.

The present result with respect to extraversion is in line with findings of Blankstein et al., 2009, who used a similar methodology (i.e. an optimized VBM approach) in an adolescent sample, and another study that showed a relation between extraversion and PFC volume in an adults sample (DeYoung et al., 2010). However, also neuroticism has been related to OFC volume (Wright et al., 2006). Accordingly, in the present study, we expected neuroticism to account for a substantial portion of the volumetric variation in regions associated with emotional perception and regulation. However, no such relation was observed. Instead, extraversion was found to be the main predictor of regional brain volume in affective brain regions. This discrepancy in findings might be due to methodological issues such as those outlined before, most importantly, this study used an optimized VBM method in a relatively large adult subject sample. Future research has to elucidate whether this lack of neuroticism - brain volume relations is a stable finding.

In the present study we examined structural correlates of extraversion and neuroticism in a cross-sectional design. Therefore, it is possible that the found correlates are not primary, but secondary to individual lifetime experiences. For instance, high extraversion is known to be associated with different lifetime experiences than low extraversion. Findings from the same NESDA study indicate that extraversion and negative life events mediate the course of depressive symptoms (Spinhoven et al., 2011), suggesting that extraversion, also defined as the tendency to engage in reward-enhancing behavior, could influence the likelihood of experiencing positive life events or how certain life events are perceived. In addition, extraversion is argued to be a protective factor for dysthemia and social anxiety rather than other anxiety disorders (Kotov, Gamez, Schmidt, & Watson, 2010). Interactions between extraversion, lifetime experiences (both positive and nega-

tive) and brain structures should be investigated as this could shed light on the development of different psychiatric disorders.

Given the augmenting evidence for a significant relation between extraversion and OFC and amygdala volumes, future research should address structural and functional connectivity of the OFC and the amygdala, and further investigate the role of the pregenual ACC in this circuitry. This might provide more insight about the trajectory from health to psychopathology, and, in doing so, identify neuroanatomical markers which relates to one's risk of developing affective disorders.

Chapter 4

Neural sensitivity to social reward and punishment anticipation in Social Anxiety Disorder.

Henk Cremers | Ilya Veer | Philip Spinhoven | Serge A.R.B Rombouts | Karin Roelofs

Submitted for publication

4.1 Abstract

Objective: An imbalance in the neural motivational system may underlie Social Anxiety Disorder (SAD). Although cognitive theories conceptually link SAD to social punishment sensitivity, current neurobiological findings have not shown evidence thereof. This study examines *social* reward and punishment anticipation in SAD, predicting increased striatal activation for punishment avoidance.

Methods: Individuals with SAD (n = 20) and age, gender and education case-matched controls (n=20) participated in a functional magnetic resonance imaging (fMRI) study. During fMRI scanning, participants performed a new Social Incentive Delay task to measure the anticipation of social reward and punishment.

Results: As expected, healthy controls showed stronger striatal activity for reward than for punishment trials. SAD participants showed a reversed pattern of effects, indicating relative increased striatal punishment sensitivity. Furthermore, in the SAD group, behavioral data showed evidence for reaction time difference between punishment and neutral trials, lower likeability ratings for punishment cues, and a correlation between anticipatory striatal activity and cue ratings.

Conclusions: SAD participants' striatal activation balance in social reward/punishment anticipation suggests an increased motivation to actively avoid social punishments. These results highlight the impact of social incentives on the motivational system in the socially anxious brain, and point to altering the reward/punishment balance and increasing reward sensitivity when treating SAD.

4.2 Introduction

Avoidance motivation is a core aspect of social anxiety disorder -SAD- (Holtforth, 2008; Neal & Edelmann, 2003). Research over the last decades has identified a dopaminergic mediated brain circuit involved in motivational processing (Haber & Knutson, 2010), largely encompassed by the ventral parts of the striatum. Anticipatory striatal activity is thought to reflect *motivational salience,* and is linked to both appetitive -reward- and aversive motivation -punishment avoidance- (Salamone, 1994). Although SAD may be associated with an imbalance in the striatal system, it has never been studied in relation to social incentives, and it remains unknown whether the striatum is more active when avoiding social punishments or when obtaining social rewards. Insight into these core motivational systems is critical in advancing the understanding of the neurobiological basis of SAD, and in providing starting points for more focused treatment of this pervasive disorder.

A recent model integrates Reinforcement Sensitivity Theory with SAD development, and highlights the role of behavioral inhibition as a temperamental predisposition in the development of social anxiety (Kimbrel, 2008). The behavioral inhibition system is linked to punishment or threat sensitivity and the motivation to avoid potentially harmful situations -harm avoidance- (Carver & White, 1994). It is thus likely that in SAD, the striatal motivational system shows differential preference for reward and punishment avoidance, either reflecting the absence of a motivational drive to obtain a reward, heightened motivation to avoid punishments or both. Several lines of brain research have indeed linked alterations in the striatal dopaminergic systems to SAD (see Freitas-Ferrari et al., 2010 for an overview). In addition, fMRI studies in behaviorally inhibited children (Guyer et al., 2006) and adolescents with SAD (Guyer et al., 2012) found overall increased activation in the ventral striatum for impending monetary rewards and punishments. This valence nonspecific increase in striatal activity was interpreted as reflecting a general motivation to avoid making mistakes. On the contrary, another study found that high shyness relates specifically to faster responses to monetary rewards but not to punishments (Hardin et al., 2006).

None of the discussed empirical findings seem immediately compatible with the suggested motivational imbalance towards stronger punishment avoidance sensitivity. Surprisingly, however, brain responses to *social* incentives in SAD have yet to be tested. To this end, we investigated brain activation in social anxiety disorder when social rewards or punishments could be actively obtained or avoided. We hypothesized that SAD participants would show greater motivation to avoid social punishments than to obtain social rewards, reflected by stronger activation in striatal regions during punishment anticipation than during reward anticipation.

4.3 Methods

Participants

This study included 20 participants with SAD and 20 healthy control participants (selected from a pool of 24 subjects to match on age, gender, and years of education) see table 4.1. Participants completed several questionnaires: Liebowitz Social Anxiety Scale (LSAS) Social Phobia Anxiety Inventory (SPAI; Turner, Beidel, Dancu, & Stanley, 1989), Brief Fear of Negative Evaluation (BFNE; Weeks et al., 2005), Beck Depression Inventory (BDI; Beck, Steer, & Carbin, 1988), the five-factor model of personality NEO-FFI (Costa & McCrea, 1992), and the Behavioral Activation and Behavioral Inhibition Scale (BIS/BAS; Carver & White, 1994), see Table 4.1. SAD participants were recruited through advertisement (n=7) and local participating treatment centers (n=8) and social anxiety websites (n=5). Inclusion criteria for social anxiety participants were an LSAS score of 60 or higher, and to meet criteria for general SAD according to the DSM-IV (1994) criteria based on the Mini-International Neuropsychiatric Interview (MINI) as a primary diagnosis. The MINI is a well validated diagnostic instrument (Sheehan et al., 1997) and took approximately 45 minutes to complete for the SAD participants. Two SAD participants had a secondary comorbid current depressive episode, while four others had a history of depressive episodes. Two of these SAD participants were on stable Selective Serotonin Reuptake Inhibitor (SSRI) use. Healthy control participants had no history of psychiatric disease or psychotropic medication use. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and written informed consent was given by all participants.

Mean (SD)				
	Social Anxiety (n=20)	Control Subjects (n=20)	F value	p-value
Age, y	29.1 (7.5)	27.7 (7.7)	0.33	0.57
Gender, male/female	11/09	11/09		
Years of education	16	16.4	0.26	0.61
LSAS	85.9 (13.9)	21.6 (13.1)	225.23	<.001
BFNE	54.3 (5.6)	36.0 (9.2)	44.59	<.001
BDI	20.5 (11.6)	5.2 (4,.4)	40.52	<.001
NEO-N	43.6 (9.8)	29.5 (6.7)	24.54	<.001
NEO-E	30.8 (6.3)	42.7 (4.8)	39.51	<.001
BIS	24.7 (3.4)	18.5 (4.2)	25.7	<.001
bas-Reward	14.9	16.6 (2.2)	5.8	0.021

Table 4.1 Participant Characteristics
Note: LSAS = Liebowitz Social Anxiety Scale, BFNE =Brief Fear of Negative Evaluation, BDI = Beck Depression Inventory. NEO-N = NEO-FFI neuroticism, NEO-E = NEO-FFI Extraversion. BIS: Behavioral Inhibition System. BAS: Behavioral Activation System.

Materials and Procedures

Participants performed the Social Incentive Delay task (SID; Spreckelmeyer et al., 2009), a variation of the Monetary Incentive Delay task (MID; Knutson, Fong, Adams, Varner, & Hommer, 2001) which was designed to measure brain activity related to social rewards. In addition, we added a social punishment condition in order to directly compare the punishment and reward conditions. Participants are cued on the possible outcome when a target detection response (button press with right hand index finger) falls within the presentation time of that target. In the social reward condition, happy faces are the outcome of a fast response (hit) and morphed faces of a slow response (miss). In the social punishment condition, the morphed faces represent a hit, while angry faces represent a miss. In the control condition, a morphed face always follows the target, regardless of whether the response was fast enough (see Figure 4.1).

The task consists of two runs, each of 72 trials. Each trial starts with a 500 ms cue: a circle for the reward condition (n=27), a triangle for the neutral condition (n=18), and a square for the punishment condition (n=27). A fixation cross is then presented for 2250-2750 ms. The combination of the two is referred to as the *anticipation period*. The target (filled white square) is presented and participants are instructed to respond as fast as possible when the target appears. To ensure that hit rate in the different conditions was similar across participants, the target duration was variable (160–500 ms) and shortened with 10 ms for the subsequent trial when the previous target was hit. The target duration was increased with 20 ms in the subsequent trial when the previous target was missed. This algorithm leads to an approximate hit rate of 66 percent. The target is followed by the *outcome* (1650 ms), after which a black screen is presented (2500 – 5000 ms). The different trial types were presented intermixed in an event-related design, with the presentation order of trial types optimized using a genetic algorithm toolbox (Wager & Nichols, 2003).

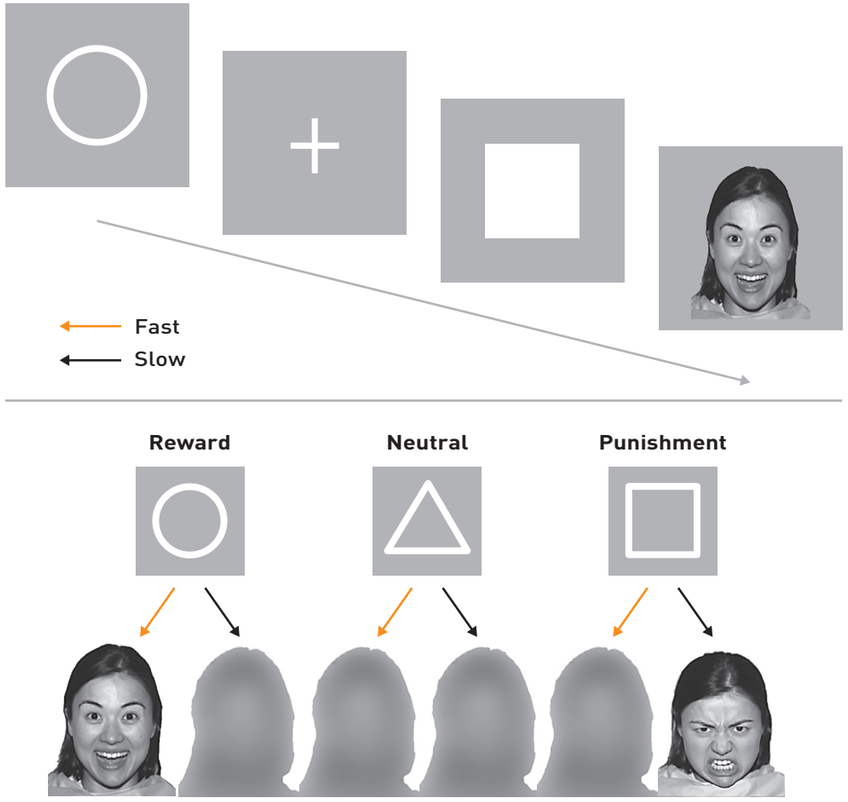

Figure 4.1 Social Incentive Delay Task.
Upper panel On each trial a cue -500ms- (corresponding to different conditions) is followed by a delay period (2250-2750 ms) after which a target is presented (150-500 ms). When the target is shown, participants are instructed to press a button as fast as possible. Depending on whether the reaction time is fast enough one of two possible feedback screens appear (1650 ms). Lower panel The different conditions with the associated feedback

The faces used in this task were taken from a standardized and validated set of facial expressions, the NIMSTIM database (Tottenham et al., 2009). Both happy and angry expression from 9 male and 9 female models were used. The morphed faces were generated using Adobe Photoshop (www.adobe.com/Photoshop).

Before the actual task, participants completed practice trials until 10 hits were obtained (irrespective of condition). After the scan protocol, participants rated how much each cue was liked on a 11 point Likert scale ranging from 0 (extremely disliked) to 10 (extremely liked), 5 being neutral.

fMRI data acquisition and preprocessing

Imaging data were acquired on a Philips 3.0-T Achieva MRI scanner using an eight-channel SENSE head coil for radiofrequency transmission and reception (Philips Medical Systems, Best, The Netherlands). Whole-brain fMRI data were acquired using $T2^*$-weighted gradient echo-planar imaging (EPI) with the following scan parameters: 298 volumes; 38 axial slices scanned in ascending order; repetition time (TR)= 2200 ms; echo time (TE)= 30 ms; flip angle = 80°; FOV = 220 × 220 mm; 2.75 mm isotropic voxels with a .25 mm slice gap. A high-resolution anatomical image (T1-weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms; TE = 4.59 ms; flip angle = 8°; 140 axial slices; FOV = 224 × 224 mm; in-plane resolution .875 × .875 mm; slice thickness = 1.2 mm), and a high-resolution $T2^*$- weighted gradient echo EPI scan (TR = 2.2 s; TE = 30 ms; flip angle = 80°; 84 axial slices; FOV = 220 × 220 mm; in-plane resolution 1.96 × 1.96 mm, slice thickness = 2 mm) were acquired for registration to standard space. Data were analyzed using FSL Version 4.1.3 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl).

The following preprocessing steps were applied to the EPI data sets: removal of non-brain tissue, spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM), grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, and a high pass temporal filter of 60 s (i.e., ≥.016 Hz). The dataset was registered to the high resolution EPI image, the high resolution EPI image to the T1-weighted image, and the T1-weighted image to the 2 mm isotropic MNI-152 standard space image (T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada). The resulting transformation matrices were then combined to obtain a native to MNI space transformation matrix and its inverse (MNI to native space).

Analysis

fMRI data

Time-series statistical analysis was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Smith et al., 2004). Explanatory variables (EV's) were included in the general linear model that modeled the anticipation of reward, anticipation of punishment and control conditions. For the outcome phase, four separate EV's were entered for hits and misses in the reward and punishment conditions, and one EV for the outcome in the neutral condition. One EV was further added to model trials where no response was given at all, while another EV was modeled for all the button presses during the target presentation to explain variance due to motor responses. Each EV was convolved with a double gamma hemodynamic response function to account for the hemodynamic delay, and in addition, the temporal derivative for each EV was included. Contrasts were generated that compared the anticipation conditions against each other (e.g. reward>neutral, punishment>neutral) and against the "implicit baseline" (reward> baseline, punishment>baseline).

The further statistical analyses were restricted to brain regions related to reward and punishment based on meta-analytic data, and a main effect of task condition (across participants). First the automated meta-analytic database Neurosynth (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011) was used to create reverse inference statistical maps related to the terms "reward" and "punishment", which were subsequently combined into one map. This statistical map was used as regions of interest in a voxel-wise analysis of the two main effects of task (reward > baseline and punishment > baseline), cluster corrected with an initial cluster forming threshold of z>2.3, and a corrected p<0.05. The two cluster corrected maps subsequently were combined in an inclusion manner (regions showing significant clusters in both the reward and the punishment condition). This combined statistical map was thus a result of regions known to be involved in reward and or punishment processing a priori, and those which showed sensitivity to this version of the social incentive delay task. Subsequently, to test for a Group x Condition interaction, mean parameter estimates were extracted for each region, condition (compared to neutral) and participant, and per region entered in a repeated measure ANOVA with group as between, and condition (reward and punishment) as within subjects factor. Grey matter values per cluster were entered as covariates.

Behavioral data

Behavioral data was analyzed with PASW Statistics, Release Version 18 (SPSS, Inc., Chicago, IL, www.spss.com) using a 2 group (SAD and controls) x 3 condition (social reward, punishment and neutral) repeated measures ANOVA on mean target reaction times. The same analysis was subsequently performed with subjective ratings of the cues as the dependent variable. In addition, correlational analyses were performed to test for a relation between behavioral variables (reaction time data, subjective ratings, social anxiety symptoms and BIS/BAS) and brain activity. A significance threshold of p<0.05 uncorrected for multiple comparisons was applied.

4.4 Results

Behavioral results

Reaction Time (RT)

Mean RT's and standard errors per group and condition are presented in Table 4.2. A repeated measures ANOVA with Group as a between-subjects and Condition as within-subjects factor showed a main effect of Condition on RT data ($F(2,76)=7.35$, $p=.001$), driven by both reward>neutral ($t(38)=3.1$, $p=0.004$) and punishment>neutral ($t(38)=2.9$, $p=0.006$) effects. There was a trend for a Group x Condition interaction ($F(2,76)=2.96$, $p=.058$). A post-hoc test revealed a significant difference between the punishment>neutral different scores in SAD compared to the control group ($t(38)=2.1$, $p=0.044$). None of the other pair-wise comparisons or simple effects were significant between the two groups (all $p>0.15$).
The adaptive reinforcement schedule resulted in the following hit rates: In the control group, the observed mean percentages of hits (± standard error) were 61.0% (±0.014 %) for reward, 61.0% (±0.014%) for punishment, and 57% (±0.019%) for the neutral condition. In the SAD group, this was 61.0% (±0.012%) for reward, 61.0.% (±0.011%) for punishment, and 54% (±0.02%) for the neutral condition. There was no significant Group x Condition interaction, and none of the conditions were significantly different between groups (all $p>0.05$).

CHAPTER 4

Mean (SE)				
	Reaction Time (ms)		Subjective Rating	
	Social Anxiety (n=20)	Control Subjects (n=20)	Social Anxiety (n=20)	Control Subjects (n=20)
Reward	240.3 (6.5)	236.0 (6.5)	7.1 (0.4)	7.1 (0.3)
Neutral	253 (6.1)	239.9 (6.9)	5.4 (0.4)	4.1 (0.3)
Punishment	238.4 (6.7)	237.0 (6.7)	4.8 (0.4)	6.0 (0.4)

Table 4.2 Behavioral Data
Note: Reaction times are in milliseconds. Subjective ratings are based on an 11-point likert scale on how much each cue, i.e. the start of a trial, was liked (0=extremely disliked, 10=extremely liked).

Subjective Ratings of cues

Mean subjective ratings and standard errors are presented in Table 4.2. A mixed ANOVA for the subjective (like-dislike) ratings of the symbols, signaling the condition at the start of each trial, yielded a main effect of Condition ($F(2,76)=33.56$, $p<.001$). Both reward>neutral ($t(38)=9.4$, $p<0.001$) and punishment>neutral ($t(38)=5.3$, $p<0.001$) were significant. A Group x Condition interaction ($F(2,76)=7.91$, $p=.001$) was also found. Subsequently, the two groups were compared on the valence-specific contrasts. Group differences were significant both for the reward>neutral ($t(38)=2.6$, $p=0.014$) and for the punishment>neutral comparisons ($t(38)=3.5$, $p=0.001$). Compared to SAD participants, controls liked both the reward and the punishment cues more than the neutral cues. Furthermore, within SAD participants, a trend was found for reward compared to punishment ($t(38)=1.9$, $p=0.067$) showing greater dislike for the punishment than for the reward symbols.

fMRI Results

The analytic procedure identified two clusters that showed a main effect of task (reward >baseline, and punishment>baseline) within reward and punishment related regions, one in the Putamen (x = -20, y =12 z = 4, k = 242) and another in the thalamus (x = 4 y = -24 z = 6, k = 409). Importantly there was

a significant Group x Condition interaction ($F(2,76)=4.26$, $p=0.046$) in the left putamen cluster, were SAD participants showed a relatively stronger activation for punishment than controls, who showed relatively stronger activation for reward (see figure 4.2). Post-hoc tests revealed that controls showed

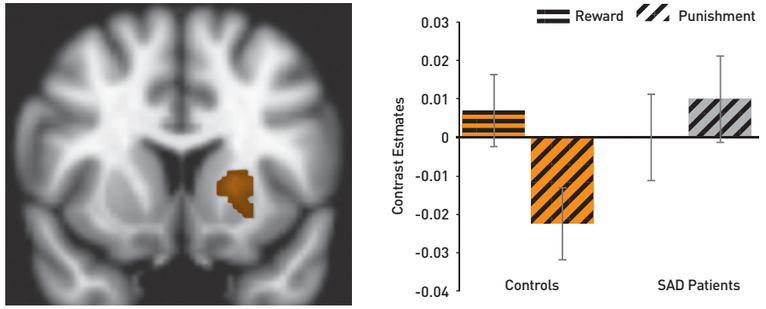

Figure 4.2 Brain activation during anticipation in the left Putamen region of interest.
Parameter estimates per group and condition compared to neutral. Left side of the image is right side of the brain. Error bars are standard error of the mean.

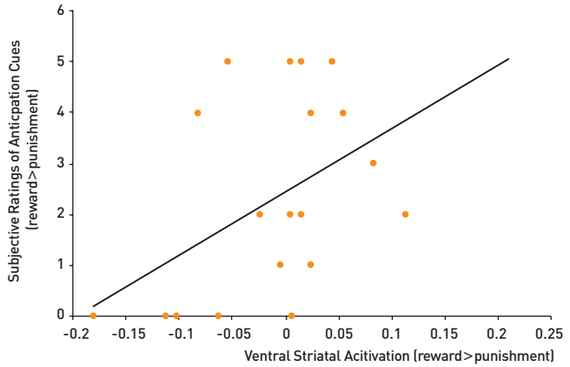

Figure 4.3 Correlation between anticipatory striatal activation (reward>punishment) and likability rating of anticipation cues (reward>punishment) in the social anxiety group

greater activity for reward than for punishment ($t(19)=2.3$, $p=0.033$), other pairwise comparisons within, or effects between groups were non-significant (all $p>0.2$). There was no significant Group x Condition interaction in the thalamus/brainstem cluster ($F(38)=0.002$, $p= 0.96$). In the social anxiety group, a positive correlation was observed between striatal difference scores (reward>punishment) and subjective liking ratings of the cues (also reward>punishment), pearson correlation: $r=0.45$, $r^2=0.20$, $p=0.047$, 95% confidence Interval: .0087-.744, spearman correlation: $r=0.52$, $p= 0.019$), see figure 4.3.

4.5 Discussion

In this study, we tested whether participants with social anxiety disorder (SAD) show differential neural activity during the anticipation of social reward or punishment. While controls demonstrated a stronger effect for reward than for punishment trials, SAD participants showed an opposite pattern of results (relatively stronger punishment avoidance sensitivity). Behavioral data displayed a trend towards a significant group by condition interaction, and post-hoc test revealed a larger reaction time difference in the SAD than in the control group when anticipating punishment compared to neutral feedback. This finding could be interpreted as a stronger motivation to avoid a punishment compared to neutral trials. However, the SAD group have a higher reaction time for the neutral trials (albeit not significantly different from the control group), which could suggest a difficulty processing ambiguous stimuli (Moscovitch & Hofmann, 2007; Moser, Huppert, Foa, & Simons, 2012). Moreover, subjective ratings also revealed a trend toward lower likability ratings of the symbol indicating punishment than the one indicating reward in SAD participants compared with controls. An additional correlational analysis indicated that increased striatal activity for reward compared to punishment anticipation also correlated with increased preference (ratings) of reward over punishment trials in the social anxiety group. Taken together, the neural and behavioral results could reflect a motivational imbalance in SAD, a state in which the usually increased motivation to obtain rewards rather than avoid punishments is shifted towards an increased relative motivation to avoid social punishments.

This study extends findings from previous studies emphasizing the role of the striatum in in behaviorally inhibited adolescents (Guyer et al., 2006) or adolescents with sad (Guyer et al., 2012). However, in contrast to these studies we showed valence specific striatal effects, arguing for a relative lack of reward and increased punishment sensitivity in sad. Our results contradict findings of a behavioral study using the mid in shy and non-shy students, that indicated that the high shyness group was more reward driven (Hardin et al., 2006). The authors suggested this finding could have resulted from a conflict in the high shyness group between pressing a button (approach) and their motivation to avoid a punishment cue. Our data suggest that this hypothesized process might not occur when the outcomes are social in nature, and are thus more closely related to the pathophysiological mechanisms in sad. The discrepancy between the current and previous findings might therefore be related to the difference in the types of incentives (monetary in previous studies vs. social in our study). Along this line, another study on a symptom specific process in sad (self-referential processing) reported increased mpfc and amygdala activity specially in response to negative self-focused comments (Blair et al., 2008). An additional explanation for the discrepancy between the current and some previous results is that the age of the study population (children compared with adults) might play a critical role in motivational preference, as sensitivity to punishment typically emerges at a later developmental stage (van Duijvenvoorde et al., 2008). More research on the current topic is needed, and it would be worth investigating whether the proposed motivational imbalance "shifts or normalizes" after a treatment which successfully increases reward sensitivity (e.g. Borgeat et al., 2009).

In line with predictions from a recent model of SAD development (Kimbrel, 2008) and empirical findings (Kimbrel, Mitchell, & Nelson-Gray, 2010; Morgan et al., 2009), SAD participants scored higher on the behavioral inhibition scale and lower on the behavioral activation scale. These findings in combination with striatal results are not only relevant for the understanding of the neurobiology of SAD, but, on a theoretical note, also shed light on the valence specificity of the reward or motivational system in the brain, which may depend on the severity of anxiety (controls versus clinical anxiety levels). Our finding relates to the ideas that dopamine reflects motivational salience, and that the (ventral) striatum codes "wanting" rather than "liking" (Berridge, 2004; 2006). It is important to note however, that the exact functional role of dopamine is still under debate. Different dopamine systems are thought to be related to motivational salience in general, while others are specific to reward only -motivational value- (Bromberg-Martin, Matsumoto, & Hikosaka, 2010; Matsumoto & Hikosaka, 2009). In line with the anticipatory affect model (Knutson & Greer, 2008), striatal regions in control participants showed a preference for reward anticipation. This model further emphasizes the relationship between anticipatory striatal activation and subjective experience (Knutson & Greer, 2008), for which we found support in the social anxiety group. More generally, striatal (dopaminergic) anticipatory activity might depend on the individual relevance (either implicitly or explicitly determined) of the upcoming reinforcer. Along this view, a recent fMRI study found support for a modulatory role of personal relevance (when valence is constant) in the ventral striatum (Carter, Macinnes,

Huettel, & Adcock, 2009). Another study found that harm avoidance scores correlated with ventral striatal activation during active avoidance of negative outcomes (Levita, Hoskin, & Champi, 2012). This study further underscores the link between striatal activation and active avoidance, which supports our interpretation that avoiding social punishments constitutes a stronger reinforcer (motivationally more salient) for SAD participants than does obtaining social rewards.

There are a few interpretational issues that need to be discussed. One point of concern is the specificity of the findings. In this version of the sid task, we opted for a large number of the same trial types to optimize our main contrast of interest (reward > punishment anticipation). This came, however, at the expense of including another control condition (for example a non-social control condition) or applying a fully balanced design, with not only congruent trials (happy faces signaling a fast response, angry faces a loss) as we have used here, but also with the incongruent trials (i.e., happy faces signaling a slow response, and angry faces signaling a fast response), as was used by Vrtička, Andersson, Grandjean, Sander, & Vuilleumier (2008). Future studies should apply these balanced designs to get a more specific view on valence differences in social incentive anticipation. In addition, a direct comparison with another anxiety patient group can increase the potential specificity of our findings. Moreover, in this study we used static faces that did not have direct personal relevance to the participants. Several studies have used dynamic facial expressions (e.g., Trautmann, Fehr, & Herrmann, 2009) which arguably have more ecological validity. This same validity argument can be made for tasks designed to increase the personal relevance of stimuli. For example, in one study participants thought they would later engage in a computer chat session with peers, whose pictures were used as stimuli in the actual fmri experiment (Guyer et al., 2008). Both adjustments could help increase the social nature of the task.

Conclusions

Whereas controls show relatively heightened striatal response to cues signaling reward rather then avoiding punishment, SAD participants show the opposite pattern of results. This relative heightened sensitivity to cues signaling social punishment suggests that they are more motivated to avoid a potential punishment. This finding is in line with theoretical models of SAD, and emphasizes that besides "passive" anxiety for social interactions, SAD participants are also specifically motivated to actively avoid negative social feedback.

Chapter 5

Dysfunctional cortical-amygdala coupling in social anxiety during the anticipation of giving a public speech

Henk Cremers | Ilya Veer | Philip Spinhoven | Serge A.R.B Rombouts
Tal Yarkoni | Tor D. Wager

Submitted for publication

5.1 Abstract

Objective: Severe stress in social situations is a core symptom of social anxiety disorder. Connectivity between the amygdala and cortical regions is thought to be important for emotion regulation, a function that is compromised in SAD. However, it has never been tested if and how this connectivity pattern changes under conditions of stress inducing social evaluative threat. Here we investigate changes in cortical-amygdala coupling in social anxiety disorder during the anticipation of a public speech.

Methods: Individuals with SAD (n = 20) and age, gender and education matched controls (n=20) underwent three "resting-state" fMRI scans before, during and after the anticipation of a public speech. Connectivity between cortical emotion regulation regions and the amygdala was tested for group x condition interactions.

Results: SAD participants, compared to controls, showed diminished connectivity (decoupling) between cortical emotion regulation regions and the amygdala during the anticipating speaking in public. Moreover, cortical-amygdala decoupling mediated the relationship between social anxiety symptoms and increase in self-reported stress.

Conclusions: The distinctive pattern of cortical-amygdala connectivity may suggest less effective cortical communication during social stress provoking situations in SAD, which could be a core mechanism underlying perceived stress.

5.2 Introduction

Social anxiety disorder (SAD) is characterized by persistent fear of social interactions. Dysfunctional emotion regulation may be at the heart of its etiology and might involve ineffective cortical-subcortical coupling. However, such coupling has not been investigated in relation to social evaluative threat, a key component in social stress, which is difficult to study naturalistically in an fMRI context on top of that. Insight into these cortical-subcortical mechanisms is critical to advancing knowledge on the neurocognitive background of SAD, and improving therapeutic interventions in this frequent and persistent disorder. Here, we test whether and how cortical-subcortical (amygdala) connectivity in SAD alters during the anticipation of speaking in public. In addition, we test whether this pattern of connectivity mediates the relationship between symptom severity and acute stress responses.

The amygdala is extensively connected to both cortical and subcortical regions (e.g. hypothalamus and brainstem nuclei, such as the periaqueductal grey and locus coeruleus (Arnsten, 2009; Ulrich-Lai & Herman, 2009). The subcortical connections are particularly important for both the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis reactions to stressors (Joëls & Baram, 2009; Ulrich-Lai & Herman, 2009); as such, the amygdala may play a coordinating role in the stress response (Arnsten, 2009; Joëls & Baram, 2009; Ulrich-Lai & Herman, 2009). The cortical-amygdala connections on the other hand, are important for regulatory processes aimed at altering (initial) stress or emotional responses (Arnsten, 2009; Feder, Nestler, & Charney, 2009). Previous PET studies in SAD have demonstrated increased amygdala activity during speech anticipation (Lorberbaum et al., 2004; Tillfors, Furmark, Marteinsdottir, & Fredrikson, 2002). However, cortical-amygdala connectivity has not been addressed, and more generally, the role of the amygdala activity in prolonged stress states is unclear (Pruessner et al., 2008; Wager, Waugh, et al., 2009b). It is possible that social stress is reflected more by a change in the connectivity pattern (van Marle, Hermans, Qin, & Fernández, 2010; Veer et al., 2011) of the amygdala than by a prolonged increase in activity.

A recent fMRI meta-analysis identified a broad set of cortical regions involved in cognitive emotion regulation, including medial and lateral prefrontal and parietal regions (Diekhof, Geier, Falkai, & Gruber, 2011). A limited capacity to adequately (voluntarily) regulate emotion responses is thought to underlie several anxiety disorders (Amstadter, 2008). Some studies have started to investigate SAD in paradigms with an explicit instruction to the participants to regulate their emotional responses

(Goldin, Manber, Hakimi, Canli, & Gross, 2009a; Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009b). However, these emotion regulation processes are clearly also important when situational demands are high, without following explicit emotion regulation instructions (Gross, 2010). For SAD, reduced (spontaneous) regulatory processes could be particularly pronounced during public speech anticipation and may relate to an increased stress or anxiety response.

Here we investigate connectivity between the set of cortical emotion regulation regions and the amygdala in SAD participants before, during and after the anticipation of giving a public speech. We hypothesize that, compared to the control group, SAD participants are characterized by less effective cortical emotion regulation of the amygdala, reflected by diminished connectivity under social stress. In addition, we approximate the activity in the cortical regions and the amygdala by rank ordering the intensity levels across the whole brain. Finally and most critically, we investigate whether cortical-amygdala connectivity mediates the relation between anxiety symptoms and increased perceived stress levels.

5.3 Methods

Participants

This study included 20 participants with SAD and 20 healthy control participants -HC-, (selected from a pool of 24 subjects to match on age, gender, and years of education) -Table 5.1-. SAD participants were recruited through an advertisement (n = 7), local participating treatment centers (n = 8) and SAD participants' web forums (n = 5). SAD participants had to meet criteria for general SAD according to the DSM-IV as a primary diagnosis (1994) based on the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1997). Two SAD participants had a secondary comorbid current depressive episode, while four others had a history of depressive episodes. Two of these SAD participants were on stable Selective Serotonin Reuptake Inhibitor (SSRI) use. Exclusion criteria were other comorbid anxiety, psychotic or substance abuse disorders. Healthy controls participants had no history of psychiatric diseases or psychotropic medication use. Participants completed several questionnaires: Liebowitz Social Anxiety Scale (LSAS; Fresco et al., 2001) and Beck Depression Inventory (BDI; Beck, Steer, & Carbin, 1988) for initial screening, and after inclusion, the Social Phobia Anxiety Inventory (SPAI; Turner, Beidel, Dancu, & Stanley, 1989), Brief Fear of Negative Evaluation (BFNE; Weeks et al., 2005), the five-factor model of personality (NEO-FFI; Costa & McCrea, 1992) and the Behavioral Activation and Behavioral Inhibition Scale (BIS/BAS; Carver & White, 1994) -see Table 5.1-. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and written informed consent was given by all participants.

Materials and Procedures

Procedure

Participants were scanned during three 7.5-minute "resting-state" (RS)–fMRI runs, in which they did not have to perform any particular task, but were instructed to just lie still, eyes closed, without falling asleep. After a first baseline run (R1, baseline), participants were instructed that a public

Mean (SD)				
	Social Anxiety (n=20)	Control Subjects (n=20)	F value	p-value
Age, y	29.1 (7.5)	27.7 (7.7)	0.33	0.57
Gender, male/female	11/09	11/09		
Years of education	16	16.4	0.26	0.61
LSAS	85.9 (13.9)	21.6 (13.1)	225.23	<.001
BFNE	54.3 (5.6)	36.0 (9.2)	44.59	<.001
BDI	20.5 (11.6)	5.2 (4,.4)	40.52	<.001
NEO-N	43.6 (9.8)	29.5 (6.7)	24.54	<.001
NEO-E	30.8 (6.3)	42.7 (4.8)	39.51	<.001
BIS	24.7 (3.4)	18.5 (4.2)	25.7	<.001
BAS-Reward	14.9	16.6 (2.2)	5.8	0.021

Table 5.1 Participant Characteristics
Note: LSAS = Liebowitz Social Anxiety Scale, BFNE =Brief Fear of Negative Evaluation, BDI = Beck Depression Inventory. NEO-N = NEO-FFI neuroticism, NEO-E = NEO-FFI Extraversion. BIS: Behavioral Inhibition System. BAS: Behavioral Activation System.

speech had to be given after the scanning session was completed, that the researchers would be the committee that would judge them on their performance, and that their speech would be videotaped for later analysis. Importantly, a topic of the speech was not yet given. This instruction was immediately followed by a second RS run (R2, speech anticipation). Subsequently, the instruction was given that participants did not have to give the public speech after all, that it was just meant to measure their initial reaction to having to give a public speech, and that after a last scan, the experiment would be finished. This instruction was followed by a third and last run (R3, recovery). Before each instruction, participants rated their stress levels on an 11-point Likert scale (See Figure 5.1 for an outline of the procedure). This three-scan stress procedure was preceded by a social incentive delay task (Spreckelmeyer et al., 2009) and structural scans.

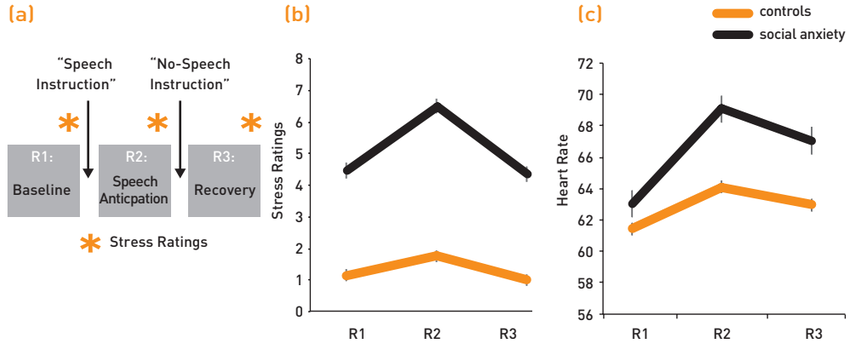

Figure 5.1 Experimental Design and Self-Report Stress and Heart Rate results.
(a) The procedure consisted of three subsequent resting-state fMRI scans. After the first scan (R1, baseline), an instruction was given that a public speech would have to be performed after the scanning sequence was finished. The instruction was followed by another scan (R2, speech anticipation) after which the instruction followed that no public speech had to be given, again followed by an fMRI run (R3, recovery). After each scan, and before each instruction a self-report level of stress was obtained on a 11point likert-scale. Heart rate was measured continuously during each scan. (b) Self-reported stress levels per scan and group. (c) Average heart rate per scan and group. All Error bars represent within subject standard error (Loftus & Masson, 1994).

Analysis

Behavioral and physiological analysis

The stress ratings at the end of each scan, before each instruction, were analyzed in a repeated measures ANOVA with Group as between and Run as within-subjects factor. During the three scans, heart rate was continuously measured using four MRI compatible ECG electrodes sampling at 500 Hz. Automatic peak detection was performed (using customized MATLAB code) on the resulting electrocardiogram (ECG) data. Two control participants were excluded from HR analysis due to excessive noise in the ECG signal. The remaining ECG data were inspected for errors in peak detection, and 0.24% of the peaks had to be manually corrected. The peak detections were used to calculate the inter-beat-intervals (IBI), which were transformed (60/IBI) to beats per minute. The resulting HR values were averaged per run, and analyzed in a repeated-measures ANOVA with group as between and run as within-subjects factor.

fMRI data

Acquisition

Imaging data were acquired on a Philips 3.0-T Achieva MRI scanner using an eight-channel SENSE head coil for radiofrequency reception (Philips Medical Systems, Best, The Netherlands). Whole-brain fMRI data were acquired using T2*-weighted gradient echo-planar imaging (EPI) with the following scan parameters: 200 volumes; 38 axial slices scanned in ascending order; repetition time (TR)= 2200 ms; echo time (TE)= 30 ms; flip angle = 80°; FOV = 220 × 220 mm; 2.75 mm isotropic voxels with a .25 mm slice gap. A high-resolution anatomical image (T1-weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms; TE = 4.59 ms; flip angle = 8°; 140 axial slices; FOV = 224 × 224 mm; in-plane resolution .875 × .875 mm; slice thickness = 1.2 mm), and a high-resolution T2*- weighted gradient echo EPI scan (TR = 2.2 s; TE = 30 ms; flip angle = 80°; 84 axial slices; FOV = 220 × 220 mm; in-plane resolution 1.96 × 1.96 mm, slice thickness = 2 mm) were acquired for registration to standard space.

Preprocessing

Data were analyzed using FSL Version 4.1.3 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The following preprocessing steps were applied to the EPI data sets: motion correction, removal of non-brain tissue, spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM), grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, and a high pass temporal filter of 100 s (i.e., ≥.01 Hz). The RS datasets were registered to the high resolution EPI image, the high resolution EPI image to the T1-weighted image, and the T1-weighted image to the 2 mm isotropic MNI-152 standard space image (T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada).

Connectivity Analysis

For the connectivity analysis, white matter, cerebral spinal fluid (CSF) and global (whole brain) signal where extracted, and entered in a regression analysis together with the six rigid-body motion parameters. The resulting residual time-series data were used for further analysis. To investigate amygdala connectivity with cortical regions involved in emotion regulation, a representative time series (first eigenvariate) was extracted from the residual data from the left and right amygdala (based on 50% probability mask from the Harvard-Oxford subcortical probability atlas, provided in FSL), and the combined set of cortical regions involved in cognitive emotion regulation (CER), based on a meta-analysis on emotion regulation (Diekhof et al., 2011). To quantify the connectivity, the two time-series for each participant and RS run were correlated, and the correlation coefficient transformed to Fisher's Z-score. These Z-values were entered in a repeated measures mixed effects ANOVA with Group as between-subjects and Run and Side (left and right amygdala) as within-subjects factors. Additionally, whole brain voxel-wise regression analyses were performed to support the initial approach. The representative CER time-series were used as regressors in a general linear model (GLM) voxel-wise analysis using FEAT version 5.98, part of FSL (Smith et al., 2004). At the subject level, contrasts were generated that tested the overall effect (across scans), the differences between the second and first scans, the second and third scans, and the quadratic effect. The resulting individual parameter estimate (PE) maps were fed into a higher-level between-groups random effects analysis (two-sample t-test). Subsequently, correction for multiple comparisons was carried out for only those voxels present in the ROI masks (left and right amygdala) using familywise error correction (FWE). In a similar fashion, voxel-wise analyses were performed with the left and right amygdala as regressor and with the CER regions as targets for small volume corrections. Effects outside our regions of interest were whole brain FWE corrected $p<0.05$.

Link between behavioral variables and changes in connectivity due to speech anticipation

Correlational analyses were performed to test the relation between the social anxiety symptoms (SPAI-SP), speech preparation related changes in brain connectivity, and self-reported stress. In order to examine whether changes in brain connectivity might drive the relation between social anxiety symptoms (SPAI-SP) (predictor) and change in self-reported stress (outcome), a mediation analysis was performed (Baron & Kenny, 1986). The predictor-outcome relation is referred to as effect *c*, and the direct effect controlling for the mediator as *c'*. Effect *a* is the relation between predictor and mediator, effect *b* the relation between mediator and outcome, and *a*b* refers to the mediation effect (the *a*b* effect tests the significance of *c* – *c'*, for which a bootstrapping procedure is applied (Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). The mediation analyses were applied to both the connectivity results from the initial analysis (CER-AMY z values), and the connectivity z-values restricted to the significant amygdala voxels from the voxel-wise regression analysis with the CER time-series as regressor. From these significant voxels in the Group x Run interaction, the times-series was extracted, correlated with the CER time-series and Fisher Z transformed.

5.4 Results

Stress Ratings and Physiological Responsiveness

The stress manipulation showed a significant Run x Group interaction on the reported stress levels ($F(2,76)= 7.2$, $p<0.001$). SAD participants reported higher stress after R2 (speech anticipation) than R1 (baseline) compared to controls ($t(38)=2.9$, $p=0.006$), see figure 5.1. The average heart rate data showed a trend towards a similar Run x Group interaction ($F(2,72)=3.07$, $p=0.052$), including a trend towards a higher score for the differences from R2 to R1 ($t(36)= 1.9$, $p=0.06$) in the SAD compared to the control group.

fMRI: amygdala connectivity

There was a significant Run x Group interaction in CER-Amygdala connectivity (quadratic contrast; $F(1,38)=4.68$, $p=0.037$), see figure 5.2. This interaction can be explained by the following pattern of effects: in controls, CER activity at baseline (R1 and R3) was correlated with reduced amygdala activity (negative connectivity) and during speech anticipation (R2), this negative connectivity was strengthened. SAD participants showed comparable negative CER-amygdala connectivity at baseline. However, in contrast to the controls, the negative coupling diminished significantly in the SAD participants during speech anticipation. No interaction of this effect with side (left or right amygdala) was observed ($p=.88$). In addition, none of the main effects were significant (all $p>0.25$). Secondly, we performed a whole-brain regression analysis, to confirm the above-mentioned findings in a voxel-wise approach. Using the CER time series as a regressor, we found an effect in the

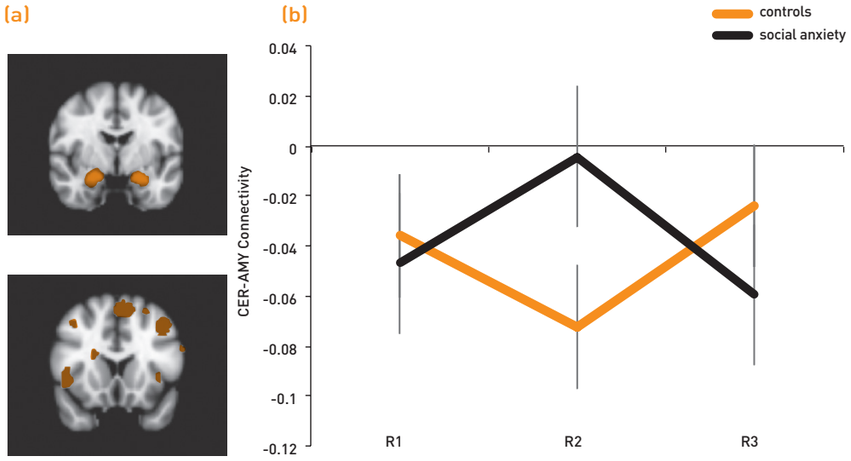

Figure 5.2 Cortical-Amygdala Connectivity
(a) Regions used for connectivity analysis, amygdala (top) and cortical emotion regulation regions (bottom). (b) Group by condition interaction on cortical-amygdala connectivity. All Error bars represent within subject standard error (Loftus & Masson, 1994)

right amygdala x = 30 / y = 0 / z = -20, $Z=3.14$, small volume FWE corrected $p<0.05$) for the quadratic contrast (comparing the baseline and recovery measurement to the speech anticipation). The analysis with the amygdala as source region did not yield any significant effect in our ROI for any of the Group x Condition interaction effects. For both analyses, no whole-brain corrected interaction effects outside of our regions of interest were found. To explore and visualize the temporal dynamics of the connectivity patterns, we used a sliding window connectivity analysis for the left and right amygdala (with the CER time-series) separately (see supplementary material).

Link between Anxiety Symptoms, Stress Ratings and Connectivity Changes due to speech anticipation

Results showed a significant correlation between social anxiety symptoms (measured with the SPAI-SP) and increases in reported stress in the social anxiety group ($r=0.48$, $p=0.048$). Using me-

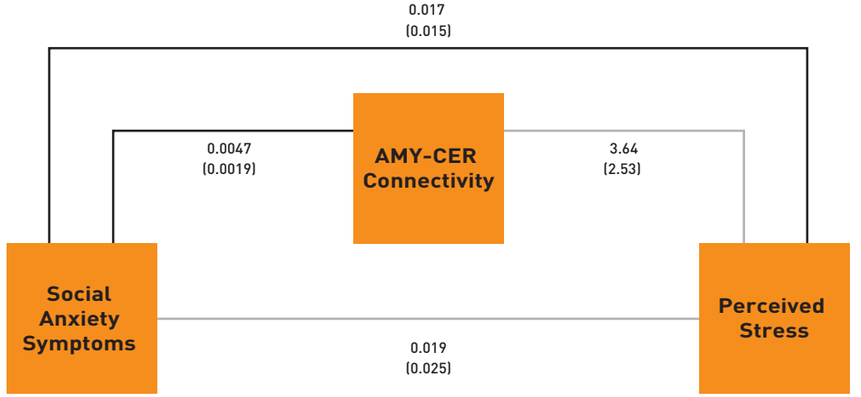

Figure 5.3 Mediation Results.
Path diagram showing the relation between social anxiety symptoms (SPAI-SP), changes in brain connectivity (run2 – run1) and changes in self-reported stress (run2 – run1). Path a is the connection between anxiety symptoms and brain connectivity, path b the connection between brain connectivity and perceived stress. Path c' is the direct connection between anxiety symptoms and perceived stress controlling for the mediator. The indirect, mediation, path (a*b) is shown as an arc connecting anxiety symptoms and perceived stress. Values for each path represent beta values with standard error in parentheses, significant paths are bold.

diation analyses with bootstrapping, we subsequently tested the indirect effect of this correlation through changes in brain connectivity. Brain connectivity (difference R2 – R1 from the voxel-wise analysis results) mediated the relationship between social anxiety symptoms and increased stress responses ($a*b$=0.016, Z=1.99, p=0.047, see figure 5.3). Path c (SPAI-SP – self-reported stress) was trend significant (c=0.035, Z=1.86, p=0.065), path a was significant (social anxiety symptoms to connectivity, a=0.0047, Z=3.079, p=0.0021) but path b (brain connectivity to change in reported stress, b=3.63, Z=1.51, p=0.13) was not. The significant positive mediation effect, and the positive values of path a and b suggest that higher social anxiety symptoms were related to "decoupling" of brain connectivity (moving from "negative" connectivity to zero or positive connectivity values), and the greater this change in brain connectivity, the greater the increase in reported stress.

5.5 Discussion

The present investigation revealed a distinct pattern of cortical-amygdala connectivity in social anxiety disorder (SAD) compared to controls when anticipating giving a public speech. The control group displayed an increase in negative connectivity under social stress. The social anxiety group however showed "decoupling" (moving from negative connectivity to no, or positive connectivity) during speech anticipation. This pattern in connectivity change may reflect failure to recruit adaptive control processes in the face of social stress. This finding shows similarities with studies that found a link between cortical-amygdala coupling and subjective or physiological responses during the instructed reappraisal of negative emotions (Lee, Heller, van Reekum, Nelson, & Davidson, 2012; Urry et al., 2006; Wager et al., 2008) and research that showed some indication of less cortical-amygdala connectivity during cognitive reappraisal in SAD patients (Goldin et al., 2009b).

The results of self-reported stress and heart rate suggest that the applied speech anticipation procedure can indeed be considered stress-inducing, and is potent in differentiating the controls from the social anxiety group. Our findings are broadly in line with various studies that have shown increases in physiological and self-reported responses to (the anticipation of) public speech in social anxiety (Blöte, Kint, Miers, & Westenberg, 2009; Davidson, Marshall, Tomarken, & Henriques, 2000; Gramer & Saria, 2007). It is of great interest that the changes in cortical-amygdala connectivity (as obtained from the voxel-wise analysis) in the SAD group mediated the relationship between social anxiety symptoms and increases in self-reported stress. Although speculative, the changes in brain connectivity may underlie the relationship between social anxiety symptoms and perceived stress. Separate cortical-subcortical pathways might be involved in the regulation or initiation of other stress reactions. For example, it has been shown that MPFC-PAG connectivity mediates HR increase during speech anticipation (Wager, van Ast, et al., 2009a) and other work has linked endogenous cortisol levels (Veer et al., 2012) and corticosteroid administration (Henckens, van Wingen, Joëls, & Fernández, 2011) to amygdala-MPFC connectivity.

Amygdala connectivity has been linked to anxiety disorders (Kim et al., 2011) and our data emphasizes the importance of amygdala connectivity for SAD. However, the results of the rank-ordered activity levels (see Supplementary Methods 1) of the amygdala do not indicate stress-related differences between the control and SAD groups (see Supplementary Results 1). The cortical emotion regulation regions showed a trend towards relatively stronger activation in the control group during speech anticipation perhaps this also points at increased cortical regulation. Our findings suggest that the connectivity specifically differentiates the controls from the SAD patients. While the role of the amygdala is well established in ambiguity detection of unpredictable or salient stimuli (Davis & Whalen, 2001; Whalen, 2007), the role of the amygdala in prolonged social stress states is currently debated, as decreases have also been reported (Pruessner et al., 2008; Wager, van Ast, et al., 2009a). Amygdala *activity* and *connectivity* may have partially different functions under certain circumstances. It is conceivable that amygdala activity (in its role of vigilance or ambiguity detector) is most strongly increased during for example face processing, and stress might increase reactivity to subsequent facial stimuli (Oei et al., 2012; van Marle, Hermans, Qin, & Fernández, 2009). However, in its link to prolonged states of social stress itself, the role of the amygdala may be better understood as integrating cortical responses on the one hand, and subcortical responses (related to ANS and HPA activation) on the other, without necessary becoming more "active or inactive".

Based on the finding that cortical-amygdala connectivity mediates the link between anxiety symptoms and increases in perceived stress, one might argue that the stress-related connectivity change is a state rather than a trait marker for SAD. Subsequently, it might be expected that after successful treatment of SAD, cortical-amygdala coupling would "normalize"; connectivity under social stress would strengthen, perhaps reflective of treatment-induced increases in successful communication between cortical emotion regulation regions and the amygdala. Previous studies have already shown that the amygdala activity during speech anticipation decreases after successful treatment (Faria et al., 2012; Furmark et al., 2005), which is thought to indicate less anxiety sensitivity. Furthermore, a recent EEG study found evidence for changes in beta-delta frequency band coupling

(thought to indicate cortical-subcortical connectivity) after behavioral treatment (Miskovic et al., 2011). It would be of great interest to test whether cortical-amygdala connectivity, as measured in our current approach, indeed normalizes after treatment, and at which rate this might occur.

Several (recent) studies have addressed resting-state connectivity in social anxiety and the results suggested diminished cortical regulation (Ding et al., 2011; Hahn et al., 2011; Liao et al., 2010; Qiu et al., 2011). However, these studies used a variety of analytic strategies, which hinders direct comparison. Although each of these findings is of interest, our study shows that "resting-state" connectivity differentially changes in SAD and control participants as a function of anticipatory stress. Several researchers have pointed at the importance of state-related changes in "resting-state" connectivity in understanding the link between (resting-state) connectivity networks and cognition (Bressler & Menon, 2010; Cole, Smith, & Beckmann, 2010). Our data-analytic approach is comparable to resting-state studies that extract representative time-series from spatial maps (based on either independent component analysis in a previous step, or on predefined masks) and then use these time-series in a regression analysis, to estimate the contribution of a network to other brain regions (Cole et al., 2010; Margulies et al., 2010). In our current study however, we departed from a set of regions not grouped by their temporal profile, but by their involvement in a certain function (cognitive emotion regulation) as identified in a meta-analysis. Our approach assumes that no cortical region in particular (necessarily) drives our findings, but rather, each node contributes to the signal of a set of cortical regions. Complex functions like emotion regulation are also most likely not to emerge from a single region, or a single connection, and the large set of regions identified by the meta-analysis on emotion regulation adds to this notion (Diekhof et al., 2011). Future research on stress responses in social anxiety could further focus for example on changes in network properties of cortical emotion regulation regions due to social stress.

Much remains unknown about the cortical-subcortical connectivity effects with respect to emotion and stress regulation; especially the timing of the stress processing is probably another crucial factor in investigating brain connectivity. For example, increases in amygdala-salience network connectivity have been observed directly after stress (van Marle et al., 2010), increased amygdala-precuneus/PCC an hour after social stress (Veer et al., 2011) and another study showed differential effects of slow genomic or fast non-genomic corticosteroid activity on amygdala response to emotional faces (Henckens, van Wingen, Joëls, & Fernández, 2010). There is also an increasing interest in dynamic changes in connectivity within a measurement period (Chang & Glover, 2009; Cribben, Haraldsdottir, Atlas, Wager, & Lindquist, 2012). The time-varying changes of cortical-

amygdala connectivity during speech anticipation hint at the possibility of stronger earlier differences between SAD and controls for the right amygdala (see Supplementary Figure 5.2). It has to be noted however, that these interpretations are speculative, and no formal statistical significance between the two groups was observed (see Supplementary Results 2). It is of interest that a recent paper, applying a paradigm similar to that applied in the current study, found time-varying effects in the occipital regions during early phase, and insula during recovery phase of social evaluative threat processing in SAD (Waugh, Hamilton, Chen, Joormann, & Gotlib, 2012).

Limitations

Although the mediation analysis results show an interesting link between social anxiety symptoms and current stress levels during speech anticipation, it does have to be noted that only the mediation results from the time-series connectivity extracted from the voxel-wise analysis (amygdala voxels) were significant. The connectivity results from the initial analysis (whole amygdala) were not significant, although the coefficients of each of the paths were in the same direction. It is possible that this is simply a result of a lack of statistical power (i.e. with a larger subject sample the results would have been significant for the whole amygdala time-series). Another possibility is that only part of the amygdala (here, maybe the basolateral nuclei) is important for the mediation effect. We do note however, that our current method of extracting one time-series over a broad set of cortical regions, in combination with the inherent problem in fMRI to accurately differentiate between activities in small adjacent structures, makes inference about the subnuclei functioning of the amygdala extremely difficult.

Another important issue in the interpretation of the data is the direction ("positive or negative") of the connectivity effect. As mentioned, EEG studies have shown some evidence for *increases* in beta-delta frequency band coupling in SAD under social stress (Miskovic et al., 2010). Since we have shown a "decoupling" between cortical-amygdala connectivity in social anxiety, these findings seem to be at odds with each other. It is important to note that the differences in techniques and analytic approaches are large, which hampers comparing these findings in any detail. In addition, one preprocessing step in our analysis, which is important to point out, is the removal (by regression) of global signal fluctuations. This procedure increases the range of correlations that can be observed between regions or networks, but it is argued that this procedure can "induce" anti-correlations,

or at least make the sign of the correlations uninterpretable (Cole et al., 2010). At the very least, we agree that our findings should be interpreted in light of the global signal regression step, and "negative connectivity" is therefore necessarily a relative value with respect to global signal fluctuations.

Conclusion

In this study, we have shown that cortical emotion regulation–amygdala connectivity changes in SAD participants in the opposite direction of that of controls, when anticipating speaking in public. The alteration in brain connectivity mediated the relation between social anxiety symptoms and increases in perceived stress. These findings suggest that social anxiety disorder participants show a diminished cortical regulation of the amygdala when anticipating speaking in public. More research is needed to test whether this potentially maladaptive change in cortical-amygdala connectivity normalizes after treatment.

5.6 Supplementary Material

S1. Rank Percentage of activity level

S1 Methods

Assessing brain activity in resting state fMRI scans is difficult because of a lack of a baseline condition within each scan to compare the activity to. Yet, In order to approximate activity within each scan, the following procedure was applied: for each participant and scan, the pre-processed data was averaged over the entire time-series per voxel. These mean values per voxel were subsequently rank-ordered across the whole brain, and transformed to a percentage (rank order/total voxels *100). As such, a value per voxel is obtained that describes the relative strength (as a percentage) of activity compared to the rest of the brain. These values were subsequently averaged per region (left and right amygdala and cortical regions) and entered in a repeated-measures ANOVA, with group as between and run as within factor. For the analysis of the amygdala, side (left or right) was additionally entered as within-subjects factor.

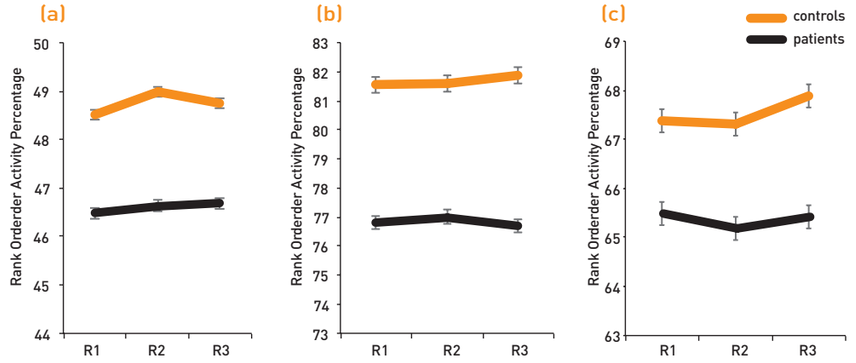

Figure S5.1 Rank percentage of activity level.
Normalized levels of activity for each group and run. (a) Cortical emotion regulation regions (b) left amygdala (c) right amygdala. Error bars represent within subject standard error.

S1 Results

For the rank-ordered percentage of the CER, a repeated measures ANOVA with group as between and run as within factor, showed a main effect of run ($F(2,76)=3,77$, $p=0.027$). The group x run interaction showed a trend towards a significant effect on the quadratic contrast ($F(1,38)=3.1$, $p=0.087$), see figure S5.1. Group showed no significant main effect ($F(1,38)=1.98$, $p=0.16$). For the analysis of the amygdala, a repeated measures ANOVA with group as between, and run and side (left or right) as within factors. Side showed a main effect ($F(1,38)=52.24$, $p<0.001$), there was no significant effect of run or a run x group interaction (both $p>0.32$). There was no significant main effect of group ($F(1,38)=1.01$, $p=0.32$

S2. Time-varying changes in heart rate and cortical-amygdala connectivity

S2 Methods

To test the possibility that connectivity between the amygdala and the cortical emotion regulation regions would vary during the course of the stress measurement (R2), and to visualize this response, we estimated dynamic changes in connectivity. This analysis was done by applying a Gaussian sliding window of 60 TR over the time-series to calculate the Pearson correlation coefficient, and employing a Fisher Z transform (see Chang & Glover, 2009, for a similar approach). The analyses were performed on the CER with the left and right amygdala separately. To subsequently test if the two groups differed in onset and duration of this connectivity or heart rate responses during the speech anticipation run, change-point estimation was applied, using hierarchical exponentially weighted moving average (HEWMA; Lindquist, Waugh, & Wager, 2007). A change point here was defined as a difference between the two groups after the baseline (the last 100 data points of the first run). To correct for multiple comparisons the HEWMA method incorporates a monte-carlo procedure, and test observed differences between the two groups to a distribution of maximum t values from randomly generated time-series of equal length.

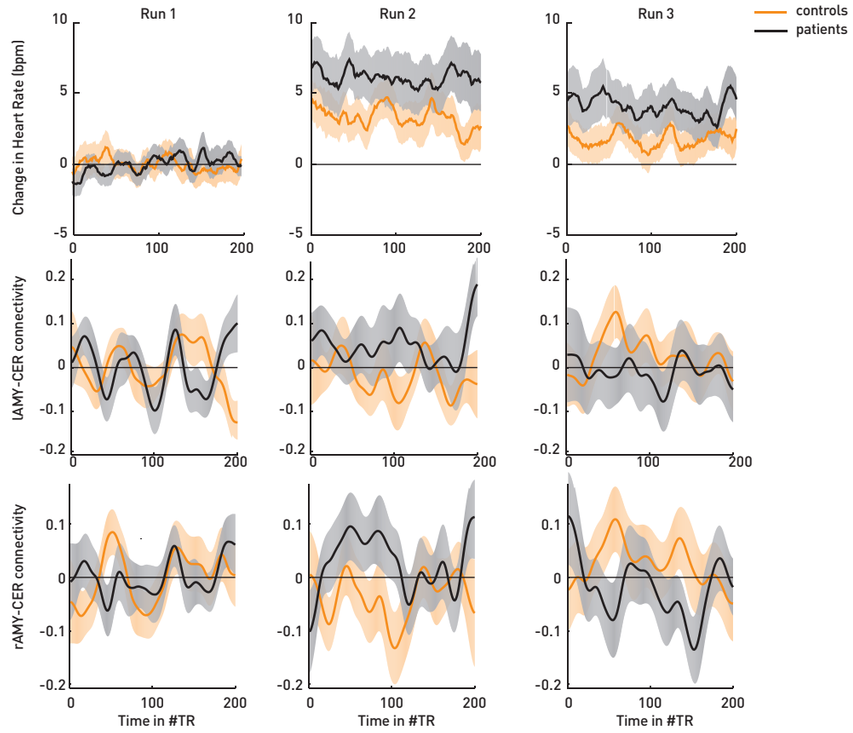

Figure S5.2 Time-Varying Estimates of Hear Rate and Connectivity
Time-varying estimates per measurement period (baseline, speech anticipation, and recovery). All values are baseline (R1) corrected. (a) Heart rate (b) Left amygdala – CER connectivity (C) Right amygdala – CER connectivity. Shaded area is standard error of the mean per group.

S2 Results

Figure S5.2 display the time-varying responses per measurement period. However, no significant change-points between the groups were observed for any of the three measurements (Heart rate, CER-lAmygdala connectivity, CER-rAmygdala connectivity, all $p>0.39$).

Chapter 6

Neuroticism and extraversion differentially modulate cortical-amygdala coupling before, during and after stress.

Henk Cremers | Ilya Veer | Philip Spinhoven | Serge A.R.B Rombouts | Karin Roelofs

In preparation

6.1 Abstract

Objective: Extraversion and neuroticism are personality traits related to experiencing positive and negative affect, respectively. In addition, they are regarded as vulnerability and protective factors for the development of affective disorders. The amygdala plays an important role in stress and emotion processing, and cortical-amygdala connections are thought to reflect emotion regulatory processes. However, we know little about the relationship between personality traits and such regulatory brain mechanisms during social stress. Here, we investigated connectivity between meta-analytic based cortical emotion regulatory regions and the amygdala during resting-state state fMRI baseline measurements and anticipation of public speaking.

Methods: Participants underwent three "resting-state" fMRI scans before, during and after the anticipation of a public speech. Brain connectivity between cortical emotion regulation regions and the amygdala, heart rate and subjective stress were investigated in relation to neuroticism and extraversion.

Results: During the anticipation of public speaking, heart rate and subjective stress levels were significantly increased. Cortical-amygdala connectivity was negative overall (across measurements) and showed a trend toward stronger negative connectivity during stress. However, personality factors modulated the connectivity patterns. Neuroticism showed a positive correlation (ranging from stronger negative values towards no, or slightly positive connectivity) with cortical-amygdala connectivity irrespective of the stress phase. Extraversion, however, showed a negative correlation with stress-induced changes in connectivity (stronger negative connectivity during stress).

Conclusions: Stronger connectivity (positive or negative) might reflect cortical regulation by the amygdala, and neuroticism may relate to tonic cortical dysregulation, while extraversion may relate to phasic cortical control during social stress. Although this interpretation is tentative, the present results offer valuable clues to the mechanisms by which personality and cortical-amygdala connectivity interact in different emotional states.

6.2 Introduction

Neuroticism and extraversion are personality traits related to experiencing negative and positive affect, respectively (Larsen & Ketelaar, 1991). These traits are also associated with the development of anxiety and mood disorders, where neuroticism is considered to be a vulnerability factor, and extraversion is thought to serve as a protective factor against the development of anxiety and depression (Bienvenu, Hettema, Neale, Prescott, & Kendler, 2007; Bienvenu et al., 2004). Yet, much remains unknown about the neurobiological mechanism of important processes of anxiety, which could link to these personality traits. Particularly, insight into the link of neuroticism and extraversion with neurobiological mechanisms of stress-inducing social evaluative threat may be critical to the advance of knowledge on the development of pathological anxiety.

The amygdala is involved in a wide variety of affective functions, including a coordinating role in the stress response (Arnsten, 2009) and emotion processing (Kober et al., 2008; van Marle, Hermans, Qin, & Fernández, 2010). Several cortical -medial and lateral prefrontal, as well as parietal- regions (Diekhof, Geier, Falkai, & Gruber, 2011) are associated with emotion regulation and are thought to influence amygdala response to emotional or stressful situations. Connectivity between the amygdala and cortical regions involved in emotion regulation can be indicative of adequate neural communication. In addition, cortical-amygdala connectivity is linked to individual differences in personality (Cremers et al., 2010) and to anxiety disorders (Kim et al., 2011). Personality traits are also linked to individual differences in stress reactivity (Tyrka et al., 2006; 2007) and emotion regulation (Tamir, 2005; 2009). Cortical-amygdala connectivity might represent a crucial neural correlate of stress coping under social threat. However, the relation between cortical-amygdala connectivity and personality traits under social evaluative threat has not been addressed.

Speaking in public is stress provoking for almost anyone to some extent, but the anxiety is typically very high in patients with social anxiety disorder. In chapter 5, we have reported on the differences in brain connectivity in SAD patients compared to matched controls. Given the link between personality factors and the development of, among others, social anxiety (Kimbrel, 2008), here, we investigate whether common variation in neuroticism and extraversion is linked to differences in cortical-amygdala connectivity during the anticipation of speaking in public in healthy controls. We expect that higher neuroticism scores are related to less regulatory capacity, and that higher extraversion scores are related to greater regulatory capacity, reflected by differential cortical-amygdala connectivity at rest compared to the anticipation of speaking in public.

6.3 Methods

Participants

This study initially included 24 healthy control participants (HC), and is part of a larger research project on social anxiety disorder, the results of which were presented in chapter 4. Participants in the current study had no history of psychiatric diseases or psychotropic medication use. Participants completed several questionnaires, of which the five-factor model of personality NEO-FFI (Costa & McCrea, 1992) was used for the current analysis, see table 6.1. One participant did not complete the NEO-FFI and has been excluded from further analysis. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and written informed consent was given by all participants.

	Mean	Standard Error	Minimun	Maximum
Neuroticism	28.52	1.44	16	44
Extraversion	43.22	0.98	35	55
Age	28.57	1.67	19	46
Gender	10 M / 13 F			

Table 6.1 **Demographic Data**

Materials and Procedures

Procedure

Participants were scanned during three 7.5-minute "resting-state" (RS)–fMRI runs, in which they did not have to perform any particular task, but were instructed to just lie still, eyes closed, without falling asleep. After a first baseline run (R1, baseline), participants were instructed that a public speech had to be given after the scanning session was completed, that the researchers would be the committee that would judge them on their performance, and that their speech would be video-taped for later analysis. Importantly, a topic of the speech was not yet given. This instruction was immediately followed by a second RS run (R2, speech anticipation). Subsequently, the instruction

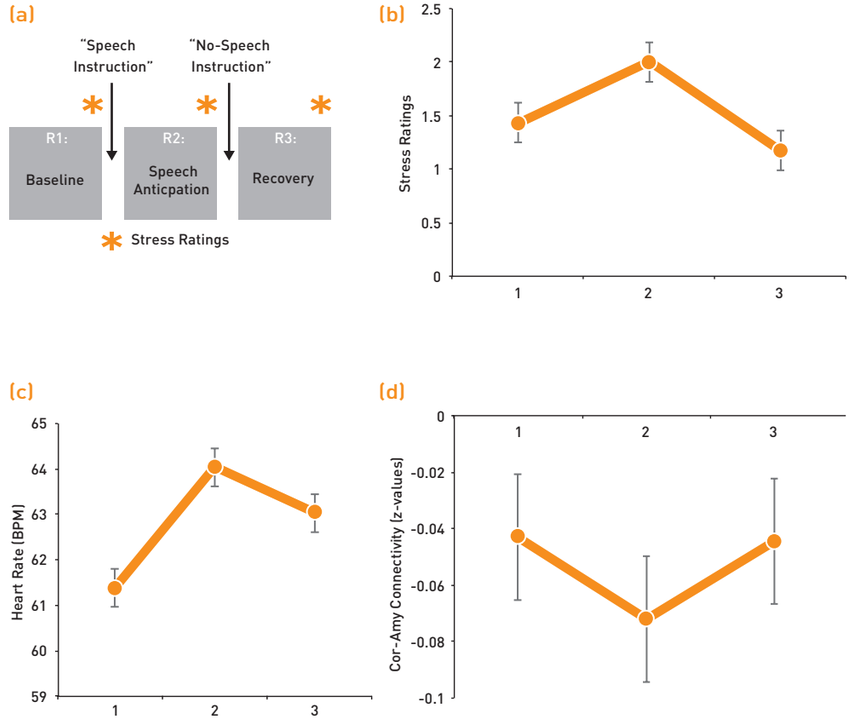

Figure 6.1 **Experimental Design and Stress responsivity**
(a) The procedure consisted of three subsequent resting-state state fMRI scans. After the first scan (R1), an instruction was given that a public speech would have to be performed after the scanning sequence would be finished. The instruction was followed by another scan (R2), after which the instruction followed that no public speech had to be given, again followed by an fMRI run (R3). After each scan, and before each instruction, a self-report level of stress was obtained on an 11-point Likert-scale. Heart rate was measured continuously during each scan. (b) Self-reported stress levels (c) Average heart rate (d) Cortical – Amygdala connectivity. Error bars represent within-subject error (Loftus & Masson, 1994)

was given that participants did not have to give the public speech after all, that it was just meant to measure their initial reaction to having to give a public speech, and that after a last scan, the experiment would be finished. This instruction was followed by a third and last run (R3, recovery). Before each instruction, participants rated their stress levels on an 11-point Likert scale (See Figure 6.1 for an outline of the procedure). This three-scan stress procedure was preceded by a social incentive delay task (Spreckelmeyer et al., 2009) and structural scans.

Analysis

Behavioral and physiological analysis

The stress ratings at the end of each scan, before each instruction, were analyzed in a repeated measures ANOVA with the different scans as within-subject factor. During the three scans, heart rate was continuously measured at 500 Hz using four MRI compatible ECG electrodes. Automatic peak detection was performed (using customized MATLAB code) on the resulting electrocardiogram (ECG) data. 0.24 percent of the peaks had to be manually corrected. The peak detections were used to calculate the inter-beat-intervals (IBI), which were transformed to beats per minute (60/IBI).

fMRI data

Acquisition

Imaging data were acquired on a Philips 3.0-T Achieva MRI scanner using an eight-channel SENSE head coil for radiofrequency reception (Philips Medical Systems, Best, The Netherlands). Whole-brain fMRI data were acquired using $T2^*$-weighted gradient echo-planar imaging (EPI) with the following scan parameters: 200 volumes; 38 axial slices scanned in ascending order; repetition time (TR)= 2200 ms; echo time (TE)= 30 ms; flip angle = 80°; FOV = 220 × 220 mm; 2.75 mm isotropic voxels with a .25 mm slice gap. A high-resolution anatomical image (T1-weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms; TE = 4.59 ms; flip angle = 8°; 140 axial slices; FOV = 224 × 224 mm; in-plane resolution .875 × .875 mm; slice thickness = 1.2 mm), and a high-resolution $T2^*$- weighted gradient echo EPI scan (TR = 2.2 s; TE = 30 ms; flip angle = 80°; 84 axial slices; FOV = 220 × 220 mm; in-plane resolution 1.96 × 1.96 mm, slice thickness = 2 mm) were acquired for registration of the EPI scans to standard space.

Preprocessing

Data were analyzed using FSL Version 4.1.3 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The following preprocessing steps were applied to the EPI data sets: motion correction, removal of non-brain tissue, spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM), grand-mean intensity normalization of the entire 4D data set by a single multiplicative factor, and a high pass temporal filter of 100 s (i.e., ≥.01 Hz). The RS data sets were registered to the high resolution EPI image, the high resolution EPI image to the T1-weighted image, and the T1-weighted image to the 2 mm isotropic MNI-152 standard space image (T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada). White matter, CSF and Global (whole brain) signals were extracted, and were entered in a regression analysis together with the motion parameters. The resulting residual data was used for further analysis.

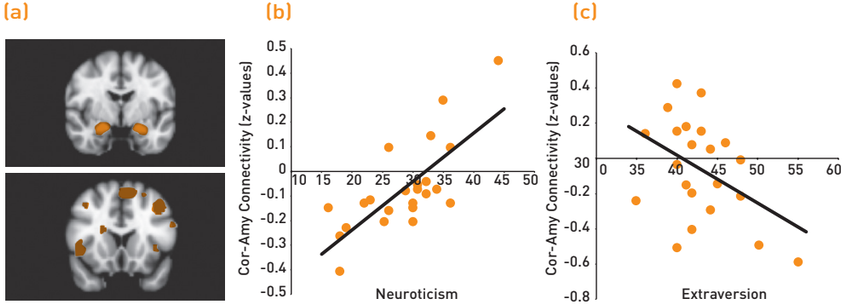

Figure 6.2 Cortical-Amygdala Connectivity and Personality
(a) Brain regions used in the analysis; amygdala (based on the Harvard-Oxford atlas) and the meta-analytic derived map of the cortical emotion regulation regions (based on Diekhof et al., 2011). (b) Correlation between neuroticism and averaged connectivity (across all conditions) (c) Correlation between extraversion and changes in brain connectivity (quadratic effect of the stress conditions).

Connectivity Analysis

To investigate connectivity between the amygdala and relevant cortical brain regions, a representative time series (first eigenvariate) was extracted from the preprocessed data from the amygdala (based on a 50% mask from the Harvard-Oxford anatomical probability atlas) and from cortical regions involved in cognitive emotion regulation (CER), based on a meta-analysis of emotion regulation (Diekhof et al., 2011) which included several lateral and medial prefrontal as well as parietal regions (see figure 6.2 for an overview). To quantify cortical-amygdala connectivity, the two time-series for each participant and RS run were correlated, and the correlation coefficient was transformed to Fisher's Z-score. The connectivity measures per run were analyzed in a repeated measure ANOVA with scan as within-subject factor.

Analyses with Personality

Each of the measures of stress reactivity (subjective stress ratings, heart rate, cortical-amygdala connectivity) were analyzed in a repeated measure ANOVA, with run as within-subject factor. The focus was on both linear and quadratic within-subject effects. The quadratic contrast effects were of specific interest because they reflect changes in the outcome variables due to the stress manipulation (comparing the effect during speech anticipation with the baseline and recovery measurements). Subsequently, to test the influence of personality on these measures, each analysis was repeated with neuroticism and extraversion as variables of interest, and age and gender as control covariates. For each significant effect, partial correlation and corresponding confidence intervals are also reported as an index of standardized effect size.

6.4 Results

Results

Stress Ratings and Physiological Responsiveness

The repeated measures ANOVA showed a significant effect of run on the reported stress levels ($F(2,36)=5.3$, $p=0.007$), see figure 6.1. A post-hoc t-test revealed a significant increase from speech anticipation (R2) compared to baseline (R1) ($t(22)=2.24$, $p=0.036$), and a significant decrease from R2-R3 ($t(22)=2.7$, $p=0.013$). Heart rate data also showed a significant effect of run ($F(2,40)=0.04$, $p<0.001$), and post-hoc t-test revealed a significant increase from R2 to R1 ($t(20)=3.81$, $p=0.001$), and a trend significance difference between speech anticipation (R2) and recovery (R3) ($t(20)=1.8$, $p=0.081$). The effects of reported stress levels and heart rates were not significant when the covariates neuroticism, extraversion, age and gender were entered (all $p>.4$), although no significant interaction or main effect of any covariate of interest was observed either (all $p> .4$).

fMRI: amygdala connectivity

There was no significant main effect of run on the CER-amygdala connectivity values ($F(2,44)=0.68$, $p=0.51$, quadratic contrast, $F(1,22)=1.2$, $p=0.28$). However, the model that included personality showed a significant effect of neuroticism ($F(1,18)=20.53$, $p<0.001$, partial correlation $r=0.66$, $p=0.001$, 95% C.I: 0.34 – 0.84) and a significant run x extraversion interaction ($F(2,44)=3.35$, $p=0.046$, quadratic effect $F(1,18)=5.09$, $p=0.037$, partial $r=-0.45$, $p=0.03$, 95% C.I: -0.73 - -0.041), see figure 6.2. Although included as a control variable, gender showed an interaction effect with scan ($F(2,36)=3.68$, $p<0.035$).

6.5 Discussion

The present study investigated the effects of the personality traits of neuroticism and extraversion on cortical-amygdala connectivity before, during, and after social stress. The results show that cortical-amygdala connectivity is differentially modulated by neuroticism and extraversion: the higher the score on neuroticism, the higher (less negative) the cortical-amygdala connectivity. Extraversion on the other hand showed an opposite and stress-specific pattern of modulation of cortical-amygdala connectivity. The higher the extraversion score was, the lower (more negative) the connectivity during the speech anticipation period was. These findings suggest that neuroticism relates to cortical-amygdala connectivity, but does not depend on the contextual demands (baseline or speech anticipation). The relation between extraversion and connectivity, however, does depend on social stress conditions. Below, we will discuss the link with current literature and possible theoretical implications.

The present investigation builds on task fMRI work that has addressed the association between neuroticism and other personality traits related to negative affect, such as harm avoidance and

trait anxiety. For example, one study showed a relation between trait anxiety and cognitive biases (Bishop, 2007), related to impoverished cortical control (Bishop, 2008). Especially interesting is the observation that the impoverished cortical control-anxiety correlation was specifically pronounced under low task-demands (Bishop, 2008). This observation is in line with our finding of less negative connectivity in participants scoring high on neuroticism, already at baseline and irrespective of stress condition. In addition, other work reported a negative correlation between anxiety-related traits and amygdala-anterior cingulate cortex (ACC) connectivity in response to negative facial expressions (Cremers et al., 2010; Kienast et al., 2008), suggesting less regulatory capacity when viewing negative faces without high task demands however. Resting-state state fMRI studies have also addressed personality and brain connectivity. For example, one study showed distinct correlations of neuroticism and extraversion with regions involved in self-evaluation and reward processing respectively (Adelstein et al., 2011). However, contrary to our observations, another RS-fMRI study found a negative correlation between harm avoidance and negative amygdala-vmPFC connectivity, which could point at more regulation at baseline associated with harm avoidance (Li, Qin, Jiang, Zhang, & Yu, 2012). It is important therefore that the current findings are independently replicated.

Based on the current findings, one may speculate on the theoretical interpretations of the association of personality with stress processing. Neuroticism showed a positive correlation with cortical-amygdala connectivity (less negative connectivity), irrespective of stress-condition, which might indicate less cortical regulation overall. Extraversion showed a negative correlation with the quadratic effect of cortical-amygdala connectivity: connectivity became a stronger negative during the anticipation of public speaking. This may suggest that extraversion relates to spontaneous cortical regulatory processes, triggered during social stressful situations, normalizing thereafter. Arguably, neuroticism is associated with high tonic stress levels, while extraversion is linked to phasic adaptions during stress. The current literature provides some evidence in this direction. Neuroticism (part of the higher-order trait of stability) is linked to high levels of baseline cortisol, yet lower cortisol response to stress (Deyoung, 2010). Extraversion, on the other hand, is thought to be part of a higher-level trait of plasticity (Deyoung, 2010) and extraversion is positively related to stress resilience (Campbell-Sills, Cohan, & Stein, 2006; Waugh, Wager, Fredrickson, Noll, & Taylor, 2008). In terms of stress coping, extraversion might be related to challenge appraisals of stressful events (perhaps a greater sense of controllability), while neuroticism is related to threat appraisals (Olff, Langeland, & Gersons, 2005). This could also form the link between extraversion as a protective factor for the development of social anxiety, which is thought to relate to less controllability (Mineka & Zinbarg, 2006). However, this proposal is tentative, and how personality traits link to the development of anxiety within individuals is unknown. Ideally, each of these factors: stress reactivity, brain-connectivity, and personality would be investigated in a longitudinal study on the development of anxiety disorders.

Heart rate measurements and stress ratings showed a significant effect of run. These findings suggest that the procedure was successful in inducing a mild form of stress across participants. However, for these two outcome measures, personality factors did not correlate with baseline or quadratic effect. A broad relation between personality and stress (coping) is well established (Sapolsky, 1996), yet the relation between different personality traits and physiological measurements of stress reactivity has produced diffuse findings. For example, one study found a correlation between openness and cortisol response while speaking in public, while neuroticism and extraversion showed interaction effects with gender on cortisol response (Oswald et al., 2006). Another study showed that there was a relation between neuroticism and stress, but this was mediated by threat appraisal (Schneider, 2004). Earlier reports did not show any relation between personality and stress response on a single task (Schommer, Kudielka, Hellhammer, & Kirschbaum, 1999), but did when data was aggregated across several measurements (Pruessner et al., 1997). Much remains unknown about the relation between personality traits and stress responses. Although speculative, the broad personality dimensions of neuroticism and extraversion might be stronger related to the neurobiological basis than to autonomic or subjective outcomes, at least in the current paradigm where a mild stressor was used. It has also been suggested that lower-level personality characteristics might be more strongly related to stress responses than higher-order traits like neuroticism and extraversion (Oswald et al., 2006). Clearly, more research is needed to further establish the relation between higher-order and lower-order personality traits and stress responsiveness and regulation.

Limitations

The relation between amygdala connectivity and personality might vary for different cortical regions (Cremers et al., 2010). In our current approach, this "fine grained" information is lost, since the signal from several cortical regions was summarized as one representative time-series. However, the current approach greatly reduces the number of tests, and hence increases the statisti-

cal power, which is very poor for between-subject studies, especially when voxel-wise regression analyses are performed and multiple comparison correction need to be applied (Braver, Cole, & Yarkoni, 2010; Yarkoni, 2009). Future research on personality and its brain correlates needs much larger study samples to elucidate the specific relation of amygdala connectivity with the different cortical regions that subserve different functions. The investigation of potential higher-order interactions, such as gender x personality interactions with brain functions, would also greatly benefit from larger study samples.

On a technical note, it is important to point out that the interpretation of the connectivity findings ("positive and negative" connectivity) heavily depends on a preprocessing step in the data analysis: the removal (by regression) of global signal fluctuations. This procedure increase the range of correlations that can be observed between regions or networks (Cole, Smith, & Beckmann, 2010), but it is argued that this procedure can "induce" anti-correlations, or at least make the sign of the correlations uninterpretable (Cole et al., 2010). At the very least, we agree that our findings should be interpreted in light of the global signal regression step, and "negative connectivity" is therefore necessarily a relative value with respect to global signal fluctuations. In addition, the interpretation that negative connectivity would reflect cognitive regulation of the amygdala supposes a directional effect, which the current analytic strategy cannot provide. In other words, the current connectivity analyses are instances of functional rather than effective connectivity (Friston, 2011).

Our findings can be regarded as broadly in line with previous task and resting-state state fMRI work on individual differences in personality, although the current paradigm and analytic approach also substantially differ on several grounds, which makes direct comparison with other literature difficult. The speech anticipation paradigm is clearly distinct from task-related approaches because the focus is on sustained differences in brain connectivity (over the course of minutes) instead of on shorter connectivity changes (task-related, i.e. over the course of seconds). Secondly, our data-analytic approach is distinct from many task-related connectivity studies, in that we obtained a representative signal from a whole set of cortical regions grouped by their involvement in cognitive emotion regulation (Diekhof et al., 2011). In a way, the current analysis bears similarity to EEG approaches in linking approach-avoidance personality traits to prefrontal lateralization (left prefrontal cortex related to behavioral approach, and right prefrontal cortex to behavioral inhibition; Davidson, 2002; Sutton & Davidson, 1997). Although the techniques and measures are certainly very different, the overlap stems from the use of a broad index of "brain functioning" in relation to higher-order personality traits. However, instead of giving evidence for a lateralization effect, our findings rather highlight the differences between extraversion and neuroticism in their relation to stress-dependent and stress-independent cortical-amygdala coupling.

Conclusion

The current study showed a distinct pattern between extraversion and neuroticism and their link to cortical-amygdala connectivity at baseline and during the anticipation of public speaking. While neuroticism positively correlated to connectivity (irrespective of stress state), extraversion correlated negatively to stress-related changes in the connectivity pattern. Since the negative connectivity found here might reflect cortical regulation, it is possible that neuroticism is linked to tonic cortical dysregulation, while extraversion is related to phasic cortical regulation. Although this interpretation is tentative, these results offer valuable clues on the mechanisms by which personality and brain mechanism interact under different cognitive and emotional states.

CHAPTER 6

Chapter 7

The power of fMRI: considerations for clinical neuroscience

Henk Cremers

7.1 Abstract

The progress in fMRI methods has been impressive during the last two decades, yet commonly raised concerns about research practices in social and medical sciences certainly apply to different fields of fMRI research also. This paper focuses on problematic issues in between-subjects fMRI studies, such as typical group x condition interactions in clinical fMRI research. It is argued that specifically, the combination of extremely low statistical power and great flexibility in data analyses hinders the interpretation of the majority of published findings. These issues will be illustrated by examples in the literature, and a simulation is presented to exemplify the consequences of underpowered sampling from different population effects. Due to the lack of statistical power, results that survive a statistical threshold can appear strong and selective, while this is an unlikely representation of the underlying population effect. In underpowered studies, stringent multiple comparison correction has the ironic consequence of making this mismatch even worse. On the other hand, due to the many options of analysis, most papers can still report "significant" results, but these may well be false positives. Within a certain research field, published results may seem misleadingly consistent (due to the flexibility in data analyses), "unnecessarily" confusing (because of sampling error in underpowered studies) or represent a complex combination of both. In addition, since most studies lack exact and quantitative replication, therefore, the value of most clinical fMRI results is at best unclear. Despite practical and financial difficulties, adequately powered studies are warranted, ideally, combined with independent, exact and quantitative replication studies.

7.2 Introduction

> *"No one ever seemed to know exactly what hypothesis testing could tell you that was at all interesting or important […]. Somewhere along the line, however, we all internalized one lesson that is entirely correct: the more you reject the null hypothesis, the more likely it is that you'll get tenure."*
>
> – Geoffrey R. Loftus, (1991)[1]

fMRI is a unique neuroimaging tool because of its ability to non-invasively obtain measurements of brain activity. Impressive progress has been made in analyses options and tools over the last 20 years (Bandettini, 2012). On the other hand, for example, the progress made in so-called biomarker research is extremely slow (Kapur, Phillips, & Insel, 2012). This was to be expected since it is a very complex and time-consuming endeavor, but the cumulative insight into the biological basis of psychiatric diseases may also simply be hindered by statistical and publication practices that have obscured true understanding of the underlying population effects.

Recent years have seen a large amount of papers that address various kinds of concerns in common fMRI data-analysis practices. Examples include the issues of non-independence in data analyses (Kriegeskorte, Simmons, Bellgowan, & Baker, 2009; Vul, Harris, Winkielman, & Pashler, 2009), of spurious brain-behavior correlation (Rousselet, 2012), of adequate testing of interaction effects (Nieuwenhuis, Forstmann, & Wagenmakers, 2011), and of multiple comparison correction (Ben-

1 Loftus, G. R. (1991). On the Tyranny of Hypothesis Testing in the Social Sciences. *Contemporary psychology*, *36*(2), 102–105.

nett, Wolford, & Miller, 2009; Nichols, 2012). While these are all very relevant topics, some broader concerns are more worrisome, such as the "undisclosed" flexibility in data analysis (Carp, 2012; Ioannidis, 2008; Simmons, Nelson, & Simonsohn, 2011), the effect of bias and low prior probability of hypotheses on the possibility of the results being true (Ioannidis, 2005) or inflated (Ioannidis, 2008), and the prevalence of underpowered studies (Sedlmeier & Gigerenzer, 1989).

Here, the issues of low statistical power (small sample sizes, where results are substantially subject to sampling error) and flexibility in data analyses (many analyses options, subject to researchers' bias) will be addressed. Specifically, the focus will be on between-subjects fMRI studies such as clinical fMRI that compare different groups (e.g. patients vs. controls). Firstly, between-subjects effects are statistically less powerful than within-subjects effects. Secondly, between-subjects studies such as clinical fMRI research often contain very small sample sizes. Thirdly, while some within-subjects effects are strong and robust (e.g., amygdala activation in response to emotional faces), this is not at all clear for many of the more complex interaction and between-subjects effects. A broader discussion on the limitations of inferences made from "standard" null-hypothesis testing, and the especially relevant topic of prior probability (or pre-study odds) of hypotheses, is beyond the scope of this paper and my competence. Many excellent contributions have been made however (for examples, see: Cohen, 1994; Falk & Greenbaum, 1995; Gigerenzer, 2004; Loftus, 1996; 1991; Meehl, 1967; Nickerson, 2000).

Several examples from the literature will be discussed to illustrate the issue of low statistical power and bias. fMRI studies on social anxiety (Freitas-Ferrari et al., 2010) will serve as an example for clinical fMRI research, but the issues are generalizable to most (or all) other fMRI research on psychiatric diseases. Another typical between subjects-effect, brain-behavior correlations, will be used as an example for simulating the consequences of underpowered studies on the mismatch with the underlying population effects. It will be argued that because of the extremely low statistical power, the flexibility in data analyses, and the absence of quantitative replication studies, the value of the majority of the published clinical fMRI results is at best unclear.

7.3 Statistical Power

> *"… less is more.*
> *Except of course for sample size."*
> **– Jacob Cohen (1990)[2]**

From the recent papers expressing criticism of fMRI data analysis practices, the topic of non-independent inferences ("double dipping" or "voodoo correlations"; Kriegeskorte et al., 2009; Vul et al., 2009) has perhaps received the most attention. The main argument of these papers was that if a subset of the dependent variables is selected (voxels) based on a threshold (i.e. "where is a non-zero effect"), one cannot make unbiased inferences on the size of the effect ("how strong is an effect"). Therefore, many brain-behavior correlations reported in fMRI studies seem large, but it has been argued that this is due to the flawed two-stage analytic procedure. In a commentary on this issue, Yarkoni (2009) pointed out that the underlying reason for the seemingly high strong values of correlation is not so much the "two-staged inference" procedure as it is the *low statistical power* (statistical power can be defined as the chance of rejecting a false null hypothesis; Cohen, 1992) typical for most fMRI studies (Yarkoni, 2009; Yarkoni, Poldrack, Van Essen, & Wager, 2010).

Concerns about the prevalence of underpowered studies are widespread in psychology and have been raised for decades (Cohen, 1962; Maxwell, 2004; Sedlmeier & Gigerenzer, 1989). An important consequence of low statistical power in combination with many independent variables is the excessive overestimation (due to sampling error) of the population effect when a result reaches statistical significance (Cohen, 1994; Gelman & Weakliem, 2009; Ioannidis, 2008; Maxwell, 2004). Research on correlations between personality traits and brain activity have been highlighted as an example of such misinterpretation of fMRI results (Yarkoni, 2009). While you may expect a broad personality trait to be moderately correlated with many brain regions, an underpowered study that applies some form of statistical threshold (multiple comparison correction) will necessarily result

2 Cohen, J. (1990). Things I have learned (so far). *American Psychologist*, *45*(12), 1304–1312.

in seemingly strong and localized effects. The same principle applies to fMRI studies on group differences. In the appendix of this paper, this issue is illustrated with a simulation study.

Clearly, population effects are not known, and the estimation of statistical power of fMRI studies is difficult because of the number of alternative hypotheses and the focus on (complex) interaction effects, which makes it difficult to make assumptions about the expected effect size. Yet it seems unlikely that many of these complex cognitive phenomena studied in fMRI and the typical group x condition effects in clinical fMRI would have a particularly strong effect in any specific brain region. It can therefore be argued that as least a rough idea of statistical power and the necessary sample size can be formed. For example, consider a between-groups fMRI study with patients and controls. Suppose you expect a moderate effect size (e.g. Cohen's d = 0.5), and use an uncorrected alpha level of p<0.001 (which is therefore still very lenient, see discussion below), then 127 participants are needed in *each* group to obtain an adequate power of 80% (Faul, Erdfelder, Lang, & Buchner, 2007). This is, of course, a lot more than the commonly used sample sizes in for example SAD research, where sample sizes range from n = 7 - 27 (Freitas-Ferrari et al., 2010).

Studies on statistical power in fMRI that recommended approximate sample sizes of n = 15-25 as being sufficient, must have done so because they assumed much higher effect sizes (Desmond & Glover, 2002). This may be realistic for some very robust within-subjects effects, but seems very unlikely for comparisons between two or more heterogeneous groups. Also, when exact power calculations are not possible, post-hoc reporting of confidence intervals gives a good indication of the "state of affairs" (Liu, 2011; Loftus, 1996). If fMRI studies would present the confidence intervals that correspond to the statistical threshold, the low precision (large confidence intervals) of the estimates from underpowered studies would immediately become apparent. Note that by presenting effect sizes and confidence intervals, it would also become obvious that when adequately powered studies (with a large sample) obtain effects that are barely statistically significant, the effect sizes are small (but measured with high precision), and the relevance ("biological significance") may be questioned.

At least two solutions to increase statistical power are theoretically straightforward: apply less stringent thresholding (Lieberman & Cunningham, 2009) or test more participants (Yarkoni, 2009). In the long run, only the latter option is of course a real solution. The problem is clearly that in practice, this is not easy at all, because of the high costs of fMRI scanning on the one hand, and for clinical neuroscience, the difficulty of recruiting sufficient participants on the other. Nonetheless, you may wonder whether these practical and financial limitations offer sufficient justification

for running underpowered studies (Yarkoni, 2009). A main cause of the low statistical power in fMRI of course stems from the many tests that are performed in the common mass-univariate approaches, and the need to apply statistical correction for multiple comparisons. Hence, any method that sensibly reduces the dimensionality of the data (e.g. multivariate approaches) will, given similar effect sizes, also greatly increase power.

7.4 Flexibility in data analyses, multiple comparisons and false positive control

> *"... there is the irony that the "sophisticates" who use procedures to adjust [their alpha error] for multiple tests […] are adjusting for a nonexistent alpha error, thus reduce their power, and, if lucky enough to get a significant result, only end up grossly overestimating the population effect size!"*
>
> – Jacob Cohen (1994)[3]

As noted, in the common mass-univariate approach of fMRI data analysis, many dependent variables are separately tested, which requires a correction for multiple comparisons (MCC) to control the false positive rate (FPR). It has been shown that a commonly applied uncorrected threshold of p<0.001 produces FWE-rates of 0.15-0.4 (Wager, Lindquist, Nichols, Kober, & Van Snellenberg, 2009). On the one hand, more stringent corrections are therefore warranted, for which various methods are available (Nichols, 2012; Nichols & Hayasaka, 2003). On the other, however, it is also

3 Cohen, J. (1994). The earth is round (p < .05). American Psychologist, *49*(12), 997–1003.

clear that stringent MCC has negative consequences, a further reduction of statistical power being the most obvious one. The emphasis on MCC is usually restricted to tests within a statistical map (single image MCC). Evidently, the FPR also depends on the number of tests (i.e. different contrast) and different analyses that are performed (more stringent MCC would not be recommended however, since it would lower already dramatically low statistical power even further). The many factors determine the FPR besides single-image MCC, therefore highlight the difficulty of true FPR control within a single study (Yarkoni, Poldrack, Van Essen, & Wager, 2010).

The great emphasis on the need for multiple comparison correction, combined with the "pressure" to obtain significant and hence, publishable results, can introduce a substantial amount of research bias. Ioannidis defined bias as "the combination of various design, data, analysis, and presentation factors that tend to produce research findings when they should not be produced" (Ioannidis, 2005). In this broad definition, bias can be anything from manipulations in the analysis to selective or distorted reporting (Ioannidis, 2005). This latter notion especially constitutes what is perhaps the most common source of bias: the many analytic options (high flexibility in analyses) and selective reporting of analyses results that are "significant" (Simmons et al., 2011). A typical example in fMRI is the use of so-called region-of-interest (ROI) analyses, i.e. restricting the statistical correction to "a priori defined" brain regions. Consider the 23 fMRI studies on social anxiety (Freitas-Ferrari et al., 2010): none applied a true whole brain statistical correction for these effects, and they either relied on small-volume corrections or on cluster correction with unrealistic assumptions about the smoothness of the noise (Bennett et al., 2009). Although when accurately applied, ROI analyses are certainly a valid method (Poldrack, 2006), it is often unclear why certain ROIs are selected (and not others), and importantly, whether this was really done before the data-analysis process started (Poldrack, 2011). For some studies, the choice for an ROI is relatively straightforward (for example, the choice for the amygdala when studying face processing) but it easily becomes a lot less clear when studying amygdala connectivity for example, where many target ROIs are possible.

ROI analyses are certainly not the only statistical practice that is potentially subject to bias. As mentioned, maybe the most likely source is simply the number of different analyses that can be performed (different preprocessing options, different task contrasts, many different types of connectivity analyses, etc.). The flexibility in fMRI analyses (Carp, 2012), and many other fields (Ioannidis, 2005), is extremely large and due to continuous development of new analytic methods, it is still increasing. While this development of analysis techniques and exploratory data analysis is essential for insight into brain function, it does mean that most (clinical fMRI) studies often produce

false positive results (Ioannidis, 2005). Therefore, even whole-brain MCC gives some, but still only vague information on the actual FPR. The reason for the probability of a high FPR in clinical fMRI has to do with the ("undisclosed") flexibility in data analyses at least as much as with the lack of "correct" use of single-image MCC. It can be argued that for a more accurate FPR estimation in confirmatory studies, some form of a pre-trial register is needed in which the analyses that will be performed are described. Such a practice is already common in clinical trials (De Angelis et al., 2004). Without any form of pre-registration of the analyses, the term "corrected" results (especially, but not exclusively, small volume corrections) should be treated with much caution.

A discussion on correcting for multiple comparisons only really makes sense in conjunction with the topics of statistical power and flexibility in data analyses. An emphasis on stringent MCC will therefore ultimately only be beneficial if it leads to true improvement in statistical practices and, ideally, to adequately powered studies. However, if a one-sided focus on single-image MCR encourages fuzzy ROI corrections or (massive) additional data exploration only aimed at obtaining a "corrected" and hence "publishable" result, it brings more harm than good to insight into brain functioning.

7.5 Replication and Meta-Analyses

> *"...we must finally rely, as have the older sciences, on replication"*
>
> – Jacob Cohen (1994)[4]

Since results from many clinical (between-subjects) fMRI studies have an unclear status (due to the unknown FPR, and low statistical power), the need for independent replications rises. Direct replications do not seem to have high priority, however, since most journal publications in psychology and neuroscience appear to be entirely directed at novel findings.

4 Cohen, J. (1994). The earth is round ($p < .05$). *American Psychologist*, *49*(12), 997–1003.

Although it may be tempting to assume that a finding with a very low p-value will probably replicate, the p-value from a statistical test is not formally indicative of the replication rate, and by simulations it is also shown to be a very vague predictor of whether an effect will be replicated (Cumming, 2008). Note that this is not necessarily specific to p-values, but a general principle of point estimations in underpowered studies, because these are extremely sensitive to sampling error (Miller, 2009). When fMRI studies do report that a finding has been replicated, most often, this refers to approximate replications, and the success of replication is determined in a very broad and qualitative way (e.g. "study A found activation in the MPFC, and so did study B"). However, this description is not very informative, and there might actually be a substantial difference between the results of the two studies. Hence, such notions offer only an imprecise reference to the overlap of effects, and so, to whether the actual effect is replicated. Therefore, it is important that replications of fMRI results should be quantifiable, for example, by using statistics similar to those applied in test-retest reliability studies, assessing the overlap between statistical maps (Caceres, Hall, Zelaya, Williams, & Mehta, 2009; Plichta et al., 2012; Rombouts, Barkhof, Hoogenraad, Sprenger, & Scheltens, 1998) of the initial and the replication studies. However, the opposite observation is also very likely: effects do not seem to replicate across studies on a similar topic, but this may be due to sampling error of underpowered studies. The results of the simulation presented in the appendix, show that each replication (which in this case is even a highly unlikely *exact* replication without measurement error) of an underpowered study (n=20, case a) will produce different results, every time the experiment is run. So even without considering measurement error or bias, several underpowered studies on a similar topic can therefore combine to a highly confusing body of literature.

A recent critical analysis of gene x environment (GxE) interactions found that only 27% percent of replication studies were significant, compared to 97% of the initial results (Duncan & Keller, 2011). The authors mentioned publication bias, low power, and low prior probability of an effect being true as likely causes of this discrepancy, and concluded that most of the initial GxE interactions should be regarded as false positives (Duncan & Keller, 2011). Ioannidis has pointed to similar problems in brain morphology studies in psychiatric patient groups, where there is an "excess significance" (Ioannidis, 2011) as well as in general, research fields that are susceptible to research bias (Ioannidis, 2005). One can only speculate what the replication rate would be for clinical or other between-subjects fMRI studies, but it seems there is little reason to be very optimistic, since each of these issues (low power, bias) most certainly apply.

While direct replication studies are uncommon, meta-analyses do offer ways to detect robust patterns of activity across different studies. In addition, meta-analyses can point at consistency in small but robust effects that single studies may overlook. For example, Kober et al. (2008) showed that the PAG and hypothalamus are robustly active during emotion processing. This finding was to be expected from the animal literature, but unobserved in most individual fMRI studies, perhaps since it is a notoriously difficult for fMRI to detect activity in the brainstem (Duzel et al., 2009). Therefore, any single study might show only small effects in these regions, which can yet be consistent enough to be detected over a large number of studies. It is clear that meta-analyses aggregate (slightly) different types of studies, and you necessarily loose spatial and cognitive domain specificity. However, it could also be argued that most cognitive processes rely on activity in a distributed set of regions, which would further advocate the focus on brain networks rather than single brain regions (Bressler & Menon, 2010; Poldrack, 2011) in mass univariate statistical tests.

While meta-analyses are very useful in separating spurious from consistent findings, the results depend on the quality and potential bias of the input (Carp, 2012). The ideal input for meta-analyses would be unthresholded statistical images, since they contain the largest amount of information and are least susceptible to research bias. Moreover, different analytic techniques are still being developed for meta-analyses also (Wager et al., 2009; Wager, Lindquist, & Kaplan, 2007), and results from different types of meta-analyses, perhaps with different inclusion criteria, may vary. For instance, it has been observed that different meta-analyses on the serotonin transporter gene and life-event interactions lead to very different conclusions, presumably because of different study inclusion criteria (allowing approximate replication studies, or not; Duncan & Keller, 2011). There are not many topics in fMRI research for which a lot of meta-analyses are available, but the lateralization of amygdala functioning in emotion processing is perhaps a notable exception. Some initial meta-analyses found that a larger number of studies reported peaks in the left compared to the right amygdala (Baas, Aleman & Kahn, 2004; Wager, Phan, Liberzon, & Taylor, 2003). This was confirmed in another meta-analyses, but when focusing on effect size, there was no difference between the left and right amygdala (Sergerie, Chochol, & Armony, 2008). The authors did find evidence for an interaction of laterality and temporal dynamics (block -for the left amygdala- or event related designs). Another study found evidence for an interaction between amygdala laterality and stimulus type -stimuli containing language are related to the left amygdala, while masked stimuli relate to a higher probability of activation in the right amygdala- (Costafreda, Brammer, David, & Fu, 2008). Other recent meta-analyses seem to suggest a consistent co-activation between the left and right amygdala (Kober et al., 2008; Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012). Despite the many available meta-analyses, confusion about the "true" population effects remains.

7.6 Conclusion

The discussed issues of low statistical power, high flexibility in data analyses and lack of quantitative replication studies are particularly troublesome for the field of clinical neuroscience, where often, small heterogeneous groups of patients are compared to a control group, and where small/medium and distributed population effects are most likely to exist. Many of the current published clinical fMRI findings should be regarded as exploratory studies, which without adequately powered replication give at best a very limited insight into the underlying neurocognitive mechanisms of a psychiatric disorder. While there are many practical and financial obstacles, much larger samples are needed to adequately represent the population effects. Ideally, adequately powered studies should be followed up by independent and quantitative replication studies.

7.7 Appendix: simulation of underpowered sampling

To exemplify the consequences underpowered studies have on the results, a simulation study was performed. Consider a brain consisting of 400 (20 x 20) brain regions, each of which can be described as a between-subjects correlation between its activity and another behavioral variable (e.g. a personality trait, or a dichotomous group variable); see figure 7.1. Two different population (n=10000) effects are considered, a "medium and distributed effect" (20% of the brain correlates with the behavioral variable at a medium effect size, r = ±0.3) and a "strong and localized effect" (8% of the brain correlates with the behavioral variable, with a strong effect size, r=±0.6). From these two population effects, 5 samples (of n = 20 or n = 100) are drawn. These samples can be thought of as independent replications of the same experiment, see figure 7.1. To illustrate the consequence of different multiple comparisons thresholds, each of the replications is presented at three different statistical thresholds: unthresholded, uncorrected ($p<0.01$), and Family Wise Error (FWE) corrected ($p<0.05$ FWE corrected). In this simulation, measurement error is not modeled, and any variation between replications is due to sampling error.

A population effect where activity in many regions is moderately correlated to the behavioral variable is considered a more likely scenario than localized and very strong effects. When a study of n=20 is performed (i.e. a sample of 20 participants from the population is drawn), a few observations can be made. Firstly, an effect that survives Family Wise Error correction is a gross overestimation of the population effect. Secondly, if an effect survives FWE correction, the results appear to be similar (local and strong) to the results obtained from a population effect that actually is a localized and strong correlation. So the (corrected) results give a very poor representation of the underlying population effects. Thirdly, each replication (5 are presented here) is very likely to give a result different from the previous replication. The statistical power for each of the present population effects, at a whole brain Family Wise Error (FWE) corrected threshold is extremely low (about 0.3%). However, in this example, there are many different regions correlated with a behavioral variable, and the power to detect *any* effect (the sum of the power of all effects) is still 25%. Fourthly, although the situation is much better for a study of n=100, still, here also the power to detect each effect at an FWE corrected threshold is only 19% (yet the power to detect any effect will be up to 100%). Fifthly, consider that these where not independent replications, but independent tests (for example an fMRI data set correlated to different behavioral variables). In this case, some of these independent tests (brain-behavior correlates) may survive ("single image") FWE correction but the true overall false positive rate (across all behavioral variables) becomes unclear, and the number of false negatives is extremely large.

CHAPTER 7

Chapter 8

General Discussion

8.1 Overview of findings

The aim of the current thesis was to investigate neurobiological mechanisms in social anxiety disorder (SAD). In this thesis, I focused on two broad sets of interconnected regions, the neural motivation and emotion circuitries that have been implicated in state and trait aspects of SAD. I studied the involvement of these circuits both in relation to stress-inducing social evaluative threat (speaking in public and social punishment anticipation) and in relation to the personality traits of neuroticism and extraversion, which are important in the development of SAD. This discussion section will first summarize the main findings of each of the different studies. Secondly, the results of the studies on neuroticism and extraversion will be discussed together (chapters 2, 3 and 6). Thirdly, the neural emotion and motivation circuitries in SAD will be considered in conjunction. In addition to the results presented in the individual chapters, here I will also explore the link between the reward/punishment processing balance (chapter 4) and social stress anticipation (chapter 5). Lastly, clinical considerations, limitations and recommendations for future research will be discussed.

Chapter 2 investigated whether neuroticism modulates connectivity of the amygdala with prefrontal regions during the processing of negative emotional facial expression. The hypothesis was tested that this relationship may depend on the specific PFC functions. The results showed that right amygdala–DMPFC connectivity for angry and fearful faces, compared to neutral ones, was *positively* correlated with neuroticism scores, which suggests that neuroticism is related to increased self-referential processing of negative facial expressions. In contrast, left amygdala – anterior cingulate cortex (ACC) connectivity for angry, fearful and sad compared to neutral faces was *negatively* related to neuroticism levels, which may argue for neuroticism-dependent decreased regulation of the amygdala.

Chapter 3 described the structural brain correlates of neuroticism and extraversion in the emotion and motivation circuits. The results indicated that extraversion was positively correlated with the volumes of the amygdala and OFC, regions important for emotion processing and regulation. Surprisingly, neuroticism scores did not significantly correlate with volume in these brain regions.

Chapter 4 focused on altered motivational processes that may be key features of SAD. Based on cognitive theories linking SAD to punishment sensitivity, we predicted increased striatal activation for punishment avoidance compared to obtaining a reward. As expected, healthy controls showed relatively stronger striatal activity for reward than for punishment trials, and SAD participants showed a reversed pattern of effects. SAD participants' striatal activation balance in social reward/punishment anticipation suggests an increased motivation to actively avoid social punishments.

Chapter 5 addressed functioning of the emotion circuit in SAD, before, during and after stress-inducing social evaluative threat. Here the focus was on the interregional connectivity of the amygdala with a set of cortical emotion regulation regions. Compared to controls, SAD participants showed diminished connectivity (decoupling) between cortical emotion regulation regions and the amygdala during anticipation of speaking in public. Moreover, cortical-amygdala decoupling mediated the correlation between social anxiety symptoms and increases in self-reported stress. The distinctive pattern of cortical-amygdala connectivity may suggest less effective cortical communication during social stress-provoking situations in SAD, which could be a core mechanism underlying perceived stress.

Chapter 6 built on the work presented in chapters 2 and 3, and investigated extraversion and neuroticism as modulatory factors in social stress processing, using the same data as presented in chapter 5. Results showed that personality modulated the connectivity patterns in the healthy control group. Neuroticism showed a positive correlation with cortical-amygdala connectivity irrespective of task phase (baseline, speech anticipation, recovery), whereas extraversion showed a negative correlation with stress-induced changes in connectivity. Neuroticism might be related to *tonic* dysregulation, while extraversion is characterized more by *phasic* increases in regulation during social stress.

Chapter 7 focused on the limitations of research findings, especially in the clinical fMRI literature. Specifically the combination of low statistical power, flexibility in data analyses, and lack of direct and quantitative replication studies hinders the interpretation of many results in the clinical fMRI literature.

8.2 Neuroticism and extraversion: correlations with neural emotion and motivation regions

Chapters 2, 3 and 6 all suggested that normal variation in the personality traits extraversion and neuroticism relates to various determinants of the neural emotion and motivation circuitries. Chapters 2 and 6 point at correlations between neuroticism and connectivity patterns linked to emotion regulation: the coupling of the amygdala with cortical regulatory regions. These connec-

tivity patterns were found in relation to the processing of negative facial expressions (chapter 2) and the resting-state condition (chapter 6). For the latter results, presented in chapter 6, the correlation between cortical-amygdala connectivity and neuroticism was observed in all conditions, both at baseline and during speech anticipation. This neuroticism-connectivity correlation was not different for the speech anticipation compared to the baseline conditions. The neuroticism and amygdala-ACC connectivity results presented in chapter 2 were observed for passive processing of negative emotional facial expression. Both the relationships between neuroticism and cortical-amygdala connectivity were therefore found either without high demands on regulatory capacity (passive face processing) or irrespective of stress conditions.

Neuroticism is linked to excessive worry (Robinson, Ode, Wilkowski, & Amodio, 2007) and is negatively correlated to stress resilience (Campbell-Sills, Cohan, & Stein, 2006). Moreover, it has been found that neuroticism is related to threat appraisal, the idea that coping resources are inadequate in proportion to stressor demands (Schneider, 2004). Interestingly, it has been shown that neuroticism is correlated to high baseline cortisol, but not to cortisol reactivity during stressful events (Deyoung, 2010). Although tentative, the latter findings suggest that those with high neuroticism appraise mildly demanding situations as threatening and show less emotion regulatory capacity. On top of that, neuroticism is unrelated to cognitive tendencies to recognize threats as they occur, and to subsequently down-regulate them (Robinson et al., 2007). Another study added that neuroticism interacted with heart rate variability (said to indicate flexibility) in predicting daily levels of stress and negative affect (Ode, Hilmert, Zielke, & Robinson, 2010). In other words, not neuroticism by itself but its interaction with "flexibility" predicted daily stress levels. Neuroticism may therefore be more strongly related to passive worry than to a lack of active (flexible) coping. Along those notions, the amygdala connectivity results presented in chapters 3 and 6 could argue that neuroticism relates to "tonic" emotion dysregulation: sustained lower emotion regulatory capacity under low-threatening situations.

Chapter 3 showed that extraversion is related to larger amygdala and OFC volumes. The finding that extraversion positively correlates with amygdala volume may seem counterintuitive considering the role of the amygdala in processes such as threat detection. However, amygdala processing has also been linked to positive emotion, and amygdala volume is associated with social network size (Bickart, Wright, Dautoff, Dickerson, & Barrett, 2011). This observation is of particular interest, because extraversion is also positively correlated with social network size (Pollet, Roberts, & Dunbar, 2011). In addition, SAD has, interestingly, been linked to smaller amygdala volume (Irle, 2010). Contrarily, the amygdala is also suggested to *increase* in size due to chronic stress (Davidson & McEwen, 2012), which seems at odds with the positive extraversion-amygdala correlation. It is possible however, that the mechanisms driving the between-subjects and within-subjects effects are different. The link between larger amygdala volume and social network size or extraversion might be explained by the suggestion that a larger amygdala enables effective processing of social cues in conspecifics (Bickart et al., 2011). The within-subject mechanisms of stress-induced increase in amygdala volume, are linked to intracellular and extracellular processes increasing dendritic spine density, although much remains unknown about the exact details of these processes (see for a discussion: Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005).

The OFC/VMPFC is associated with both reward processing and emotion regulation (Kringelbach & Rolls, 2004; Milad & Rauch, 2007; Rolls, 2004). The positive correlation between extraversion and OFC volume (chapter 3) may relate to enhanced reward processing and/or increased emotion regulatory functions. With respect to the interpretation of stronger reward processing and extraversion, it is surprising however that the striatal reward/punishment effects presented in chapter 4 did not correlate significantly with extraversion. The correlation between extraversion and increased negative cortical-amygdala connectivity during stress-inducing social evaluative threat (chapter 6) fits with the notion of enhanced emotion regulatory capacity for people high in extraversion. Moreover, extraversion is linked to stronger resilience and coping (Carver & Connor-Smith, 2010) and a sense of control after adverse events (Affleck & Tennen, 1996). Greater emotion regulatory capacity and greater reward sensitivity might buffer (raise the threshold) against the negative consequence of stress or adverse events, and extraversion is related to both. Contrary to neuroticism, extraversion is related to challenge appraisal and the belief that coping skills are sufficient for stressful demands (Schneider, 2004). Since extraversion was related to the increase in negative connectivity during speech anticipation, it may suggest a more phasic regulatory role, active in high but unrelated to low-threatening situations. The correlation between extraversion and greater volume within emotion and motivation circuitry regions perhaps indicate an increased "readiness" for high demanding or threatening situations.

In sum, our data suggest that broadly, neuroticism and extraversion are differently related to the neural emotion and motivation circuitries. Whereas neuroticism may be more linked to tonic (dys)regulation, extraversion might be associated to greater reward sensitivity and phasic increase in emotion regulatory functioning during high-demanding situations. A combination of low extraversion and high neuroticism is found to be a vulnerability factor for the development

of SAD and other anxiety disorders as well as for depression (Bienvenu, Hettema, Neale, Prescott, & Kendler, 2007; Bienvenu et al., 2004). The proposed differing relation between neuroticism and extraversion with the motivation and emotion circuitries might shed light on the underlying neurobiological mechanism related to this vulnerability.

8.3 SAD and the neural emotion and motivation circuitries

Correlation between striatal reward/punishment balance and social stress processing

As was mentioned in the introduction, the motivation and emotion circuitries are linked in several ways. For example, it is has been found that acute stress leads to increased dopamine levels (Arnsten, 2009; Kienast et al., 2008; Mizrahi, 2010; Pruessner, Champagne, Meaney, & Dagher, 2004), and that stress is linked to addiction (Koob, 2008; Ulrich-Lai & Herman, 2009). On the other hand, a motivational balance towards higher reward sensitivity may buffer against stressful events, and could be regarded as an indication of stress resilience (Degnan & Fox, 2007; Masten, 2001). For example, a recent study showed that reward sensitivity, measured by striatal responses to monetary incentives, is in turn associated with less susceptibility to stress (Nikolova, Bogdan, Brigidi, & Hariri, 2012). In addition, psychiatric disorders such as depression and posttraumatic stress disorder are characterized by reduced reward sensitivity (Feder, Nestler, & Charney, 2009).

Here, we investigated whether the striatal reward/punishment balance (presented in chapter 4) was predictive of various determinants of the stress response (presented in chapter 5): heart rate increase, subjective ratings and cortical-amygdala connectivity. This was done by simple correlations between the striatal activity balance (reward vs. punishment) and stress procedure related effects (i.e. the quadratic effect: speech anticipation condition simultaneously compared to both baseline and recovery). Figure 8.1 presents the overall results across participants, (black line) and separately, for the control group (orange) and social anxiety group (grey). Most notable is that the striatal reward/punishment balance negatively correlated overall with subjective stress responses (r=0.308, p=0.036) and heart rate increases (r=0.351, p=0.027). Both these effects were trend significant in the social anxiety group (p=0.057 and p=0.063), but not in the controls (p>0.6). The relation with cortical-amygdala connectivity showed a similar pattern but was non-significant (overall: r=-0.244, p=0.129, SAD: r=-0.356, p=-0.124, controls p=0.82). These negative correlations between striatal reward/punishment balance and stress outcome suggest that, especially in the social anxiety group, the more an individual is punishment avoidant rather than reward motivated, the more stress reactive he or she is to the speech anticipation procedure.

The analyses could possibly be extended to test for example if the same cortical regions that regulate reward anticipation (PFC-VS connectivity) during the reward task are predictive of emotion regulation during speech anticipation (PFC-amygdala connectivity) on a between subject level (Mennes et al., 2010) or perhaps even on a within subject level (Mennes et al., 2011). Moreover, the function of both networks within the task paradigms could be further explored. For instance, there may be links between anticipatory striatal activity and subsequent amygdala activity (during face processing) in the social incentive delay task. These current results give some indication of a link between the motivational balance and stress sensitivity, and fit with idea that the two circuitries are interrelated, and that both are potential important neural mechanisms of vulnerability and resilience to social anxiety.

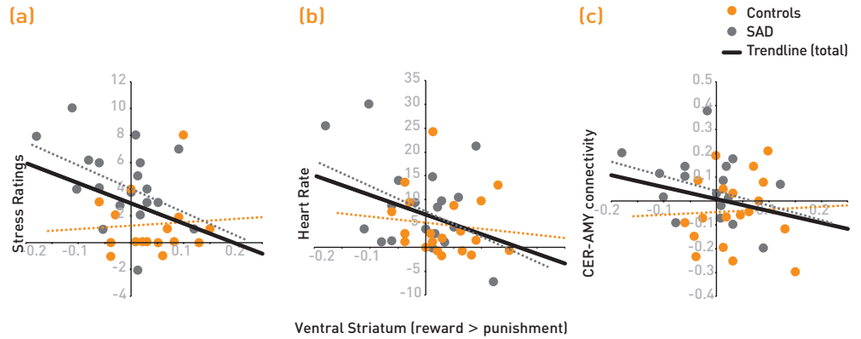

Figure 8.1 Correlations between Striatal Activity and Stress responses.
Striatal reward/punishment balance (chapter 4) is correlated with stress outcome (quadratic effects: speech anticipation condition simultaneously compared to both baseline and recovery) presented in chapter 5: (a) stress ratings (b) heart rate and (c) cortical-amygdala connectivity.

Data-analytic considerations

The analytic approaches in chapters 4-6 were mainly chosen to reduce the number of statistical comparisons to one critical outcome variable. This was done by (weighted) averaging over voxels in one broad region (chapter 4) or even several ones (chapters 5 and 6), both with the goal to increase statistical power (see chapter 7). These procedures inevitably come at the expense of potentially obscuring functional differences among various brain regions and other complexities within these neural circuitries. This is perhaps most apparent for the cortical regions involved in emotion regulation of which a single time series was extracted (chapters 5 and 6). Here, I will consider in more detail some cortical and subcortical regions and pathways that are important for SAD.

The ventromedial prefrontal cortex (vmPFC) is perhaps the core region for emotion regulation. A recent emotion regulation meta-analysis did indeed find the vmPFC to be involved across different forms of emotion regulation (placebo, fear conditioning and cognitive emotion regulation; Diekhof, Geier, Falkai, & Gruber, 2011). In a broader perspective, its function has recently been described as reflecting affective meaning (Roy, Shohamy, & Wager, 2012). The authors argued that the vmPFC is not so much involved in the affective responses per se, but critical when affective responses are shaped by conceptual information about specific outcomes (Roy et al., 2012).

In animal research, the vmPFC is often divided into the infralimbic (IL) and prelimbic (PL) subregions, which contain differential connectivity patterns, and are differentially related to initiating and regulation of stress or fear responses (Maier & Watkins, 2010; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011; Ulrich-Lai & Herman, 2009), although the exact link is not as clear as is sometimes suggested (Vertes, 2006). The subdivision of the ventral and dorsal MPFC is thought to be the human homologue of the IL/PL (Wager, van Ast, et al., 2009a; Wager, Waugh, et al., 2009b). The vmPFC and DMPFC were respectively found to be negatively and positively correlated to heart rate responses under social evaluative threat, which was mediated by several subcortical regions (Wager, van Ast, et al., 2009a; Wager, Waugh, et al., 2009b). We investigated the possibility that SAD could be characterized by dysfunctional responses in one, or both of these pathways, but no clear-cut evidence was found (results not presented). However, despite the many challenges of integrating autonomic physiological and BOLD fMRI responses (Iacovella & Hasson, 2011), neural pathways involved in the regulation of autonomic and HPA axis responses are a very relevant topic in neurobiological research on anxiety disorders.

The subcortical regions of the neural emotion and motivation circuitries can also be divided into anatomical and functional discrete subunits. The amygdala, for example, contains the basolateral and centromedial subnuclei which have very separate patterns of connections (Price, 2003), and play distinct roles in fear expression and fear conditioning (Sierra-Mercado et al., 2011; Sotres-Bayon & Quirk, 2010) and anxiety behavior (Tye et al., 2011). For example, a recent optogenetics study showed that stimulation of the BLA terminal in the CEA reduced anxiety-like behavior (Tye et al., 2011). In fMRI studies, functional connectivity differences of these main amygdala subnuclei have also been investigated (Roy et al., 2009), even in relation to anxiety disorders (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009). However, distinguishing connectivity patterns from neighboring time-series (e.g. for amygdala subnuclei) can still be regarded as challenging. With respect to the motivation circuit, the striatal effects presented in chapter 4 also encompassed different striatal regions (nucleus accumbens, putamen and caudate nucleus), which also perform partly distinctive functions within the processing of incentives (Haber & Knutson, 2010).

The amygdala also has important connectivity pathways to other subcortical structures which are relevant for stress responses. It is perhaps of interest to note that in chapter 5, amygdala-PAG and amygdala-hypothalamus connectivity differences between the SAD and control groups were observed at a more lenient threshold ($p<0.005$ uncorrected). These effects were in the opposite direction of the cortical-amygdala results: the SAD group showed enhanced positive connectivity between these subcortical regions during social stress, compared to the baseline measure. This pattern of effect seems of great interest because these regions have been linked to threat (freeze responses) and hormonal stress response (HPA axis). Therefore, future research could be directed at considering the possibility that social stress "shifts" amygdala connectivity in SAD to less cortical and stronger subcortical connectivity.

Lastly, a dichotomy between cortical and subcortical regions as reflecting emotion regulation and reactivity might need some reconsideration. It has been argued that, for example, processing in the cortex is not slower than in the amygdala, and salient visual information does not 'bypass' the prefrontal cortex (Pessoa & Adolphs, 2010). Furthermore, the cortex plays a role in emotion generation, and at the same time the amygdala has its role in cognition (Pessoa, 2008). From a theoretical standpoint it has also been argued that the distinction between emotion generation and regulation is not clear-cut, and varies widely for different emotion theories (Gross & Barrett, 2011). Furthermore, it has been shown that it is technically difficult to prove a causal relation between different brain regions with BOLD-fMRI (Smith et al., 2010), and consequently, it is therefore

fundamentally problematic to show whether for example the amygdala influences the cortex, the cortex the amygdala, or both. In combination with the finding that a wide range of (interacting) regions is involved in emotion (Kober et al., 2008) and emotion regulation (Diekhof et al., 2011), each of these issues may call for different analytic strategies, such as network approaches that better capture the many complex interactions between cortical and subcortical regions (Bressler & Menon, 2010; Bullmore & Sporns, 2009; Menon, 2011).

Clinical considerations

In line with the findings on personality differences, we also found evidence for altered functioning of the neural motivation and emotion circuitries in social anxiety disorder. The results of the two studies presented in chapters 4 and 5 suggest that SAD participants show both a striatal activity balance towards avoiding punishment as well as less effective cortical-amygdala neural communication under social stress. As discussed above, there is some initial evidence that these effects are related, or at least that the striatal findings are correlated with determinants of the stress response.

The results demonstrate that under mild threat (the possibility of negative feedback), SAD participants are still more motivated to avoid, especially when this is an explicit part of the task procedure. However, in a perhaps more threatening situation (speech anticipation), where active avoidance was not an explicit part of any task, cognitive regulatory mechanisms may not function adequately. Along the proposed notion of a relation between threat appraisal and neuroticism, SAD patients might (either consciously or unconsciously) evaluate both the upcoming negative feedback and speaking in public speech as threatening. In the "milder threatening" social incentive delay task, SAD participants are characterized by sufficient motivation to avoid a punishment (although it has been proposed that social anxiety is related to approach-avoidance conflicts in such active punishment avoidance situations; Hardin et al., 2006). During speech anticipation, however, the threat appraisal and the lack of control may result from insufficient regulatory functioning. Contrary, controls may not appraise the social punishment anticipation as threatening and hence are not necessarily motivated to avoid it, or, they are motivated but this is because of challenge appraisal. During the speech anticipations, controls may either not appraise the situation as threatening, or perhaps even appraise it as challenging.

Our current findings of relative striatal punishment sensitivity and diminished cortical-amygdala connectivity in SAD can complement theoretical models of the development of anxiety disorders. For example, the low perception of control in social situations has been highlighted as an important feature of SAD (Hofmann, 2005; Mineka & Zinbarg, 2006). In addition, higher punishment avoidance compared to reward motivation may reflect another crucial determinant in susceptibility for SAD (Degnan & Fox, 2007). It is important to emphasize that active avoidance of punishment may clearly be adaptive in many situations, whereas an exaggerated tendency to avoid, such as in the case of social anxiety, can be detrimental (Holtforth, 2008). For example, the active avoidance of social interactions may prevent natural social stress habituation processes. Also, we found preliminary evidence that punishment sensitivity and stress reactivity are correlated, which may further underscore the integrated role of both the neural emotion and motivation circuitries in resilience (Feder et al., 2009) as well as the relevance of both emotion (reactivity and regulation) and motivational functions for SAD (Degnan & Fox, 2007).

Another potential avenue for future research is to consider whether the functioning of the motivation and emotion circuitries in SAD normalizes after treatment. Several studies have started to address the effects of pharmacological and psychological treatment, and hint at this possibility. For instance, a recent study showed increased dopamine transporter binding after SSRI treatment in SAD (Warwick et al., 2012). Other findings suggest that the amygdala response in stressful public speech situations normalizes after SSRI treatment (Faria et al., 2012; Furmark et al., 2005). Lastly, recent studies have shown that mindfulness training increases activity in several parietal and cortical regions, and improves emotion regulation functions in SAD (Goldin & Gross, 2010; Goldin, Ziv, Jazaieri, Hahn, & Gross, 2012).

The observation that variations in neuroticism and extraversion which are related to SAD correlate with several determinants of the neural emotion and motivation circuitries, underscores the importance of a dimensional view of anxiety disorders (Goldberg, 2000). In a dimensional view, psychiatric disorders are regarded as the tail of a continuum rather than as a distinct category. Current DSM classifications do not capture such dimensionality well, and in addition, the diagnostic criteria for social anxiety disorder are still a matter of debate (Bögels et al., 2010). Moreover, an interesting novel network approach has further highlighted the co-occurrence of psychiatric symptoms, and emphasizes both the complex interrelated structure of these symptoms and the fuzzy nature (large symptom overlap) of many of DSM classifications (Borsboom, Cramer, Schmittmann, Epskamp, & Waldorp, 2011; Cramer, Waldorp, van der Maas, & Borsboom, 2010). Although some categorical distinction may be relatively clear (Haslam, 2003; Meehl, 2006), most DSM classifications in this network perspective were considered arbitrary (Cramer et al., 2010).

Tension seems to exist between the goals of using the DSM as an instrument to guide treatment and using it as a classification system for (brain) research into psychopathology (Goldberg, 2000). For instance, when determining a treatment plan, it may be helpful to consider whether a patient is primarily anxious or depressive. Yet comorbidity is the rule rather than the exception for most psychiatric disorders (Kessler, Chiu, Demler, Merikangas, & Walters, 2005), and for example excluding comorbidity in a (neurobiological) study of a certain disorder seems to result in an unrepresentative subsample of the wider clinical population. For instance, SAD is strongly related to major depression symptoms, and you may wonder whether it is possible or even sensible to aim at distinguishing symptoms of social anxiety and depression when they are both closely interrelated with social anxiety severity. With respect to the results obtained in the current research, it is also difficult therefore to assess how specific our findings are to SAD. Both the emotion and motivational neural circuitries, and neuroticism and extraversion are implicated in many other anxiety disorders and depression. However, we also have to consider that some diagnostic criteria may have a clear biological basis. One may for example speculate that while SAD and depression are both characterized by altered striatal functioning, to some extent, depression is stronger related to a lack of reward sensitivity while SAD relates to a stronger motivation to avoid punishment (at least for social punishments).

While a dimensional approach to psychiatric disorders may be beneficial for neurobiological studies, it is also important to emphasize that just a few basic (trait) dimensions are clearly insufficient to describe the many differences between individuals in psychopathology. For example, the same levels of social anxiety across individuals can have very different impact on the impairment of daily functioning (Rapee & Spence, 2004; Stein & Stein, 2008). Considering additional relevant dimensions rather than a categorical view may better capture these individual differences. For example, the discussed network approach to psychiatric symptoms would, as the authors themselves noted (Cramer et al., 2010), benefit from including resilience factors that buffer individuals from the occurrence of symptoms and a causal spread to other symptoms. In line with Degnan & Fox (2007), we would argue that the reward/punishment motivational balance and stress reactivity and/or controllability (with the proposed respective neural underpinnings) might form good candidates for resilience factors in models on social anxiety.

8.4 Limitations and consideration for future studies

Longitudinal Research

The results presented in the current thesis are all based on cross-sectional data. We have thus observed cross-sectional evidence for the relation between SAD, personality traits and the neural emotion and motivational circuitries. However, these neurobiological systems and the functions they underlie, also interact within individuals over time. Crucial questions about such intra-individual (causal) mechanisms cannot be answered from our data. Moreover, it has been pointed out that the mechanisms that drive between-subjects effects, can be very different from the mechanism that cause between-subjects mechanisms (e.g. Hamaker, Nesselroade, & Molenaar, 2007). An ideal study would take both between and within subjects effects into account, and obtain fMRI measurements over several time points. In that case, it would also be of interest to consider whether and how the functioning of the emotion and motivation neural systems relates to symptom severity, for instance, as a result of treatment. It is of note that the NESDA project does have longitudinal data that lend itself for some of such analyses.

Task design and analyses

The tasks used in chapters 4-6 to investigate the emotion and motivation circuitries were specifically developed to probe processes relevant for social anxiety: the anticipation of a potential social punishment and a public speech. However, the paradigms are also relatively novel, and need further investigation. Chapter 4 already discussed some possible adjustments in the social incentive delay task to increase the social nature of the task. Another possibility to consider is that the differences in reward/punishment sensitivity may be clearer as so-called adaptation effects (Etkin, Egner, Peraza, Kandel, & Hirsch, 2006), where behavior and neural activity in the current trial is modeled as a function of the previous trial. One may for example expect that social anxiety patients are more affected (e.g. show more motivation to avoid a punishment) by the current trial when the previous one resulted in a loss (for example, an angry face in the punishment condition). Although such an analysis was considered, the task would need a lot more trials per condition to adequately model these more complex interaction effects.

Another consideration in the speech anticipation paradigm (chapter 5) relates to the temporal differences in emotional reactivity and regulation responses between individuals. For example, the

difference in stress responses between controls and SAD may perhaps be clearer as "recovery speed" *after* the onset of a stressor than the "average" response *during* a stressor. These potential recovery differences could be captured by time-varying estimates of brain connectivity and physiology. Initial exploratory analyses showed no significant differences between the SAD and control groups (supplementary material chapter 5), nonetheless, time-varying responses remain a very relevant research topic for the investigation of differences between controls and anxiety patients in their stress responses.

For many fMRI tasks paradigms, little is known nor consistently found, on test-retest reliability. A recent study showed that test-retest reliability is reasonable for a monetary reward task (Plichta et al., 2012), yet a previous study found low test-retest reliability for a similar task (Fliessbach et al., 2010). Information on the reliability of the social incentive delay task has not yet been established, whereas this would be essential for longitudinal studies. With respect to stress processing in SAD, an unusual, but highly relevant recent study investigated test-retest reliability of EEG and physiological measures during public speech in SAD (Schmidt et al., 2012). The results showed medium to large correlation between two subsequent time points of various cardiovascular and EEG measurements. Ideally, similar reliability metrics would be obtained for fMRI connectivity measurements in SAD.

Larger samples and data reduction

The problem of the prevalence of underpowered studies in clinical fMRI research was discussed in chapter 7. For mass-univariate statistical approaches, much larger samples are needed to obtain adequate statistical power. Multi-center studies, such as the NESDA project (chapters 2-3) seem ideally suited for this. However, it is also important to consider that such larger studies do tend to use very general experimental paradigms (e.g. passive emotional facial expression task), aimed to be relevant for many psychiatric conditions. These paradigms are clearly not best suited to investigate specific symptoms, and may not elicit particularly large between-groups effects, which would theoretically counteract the goal of increasing statistical power by including more participants. Moreover, large multicenter studies tend to coincide with an extremely large number of statistical tests, which means that the false positive rate becomes largely uncontrollable. Therefore, notwithstanding such more adequately powered multicenter studies, replication studies (or split-half analyses, statistical power allowing) remain imperative.

In many clinical fMRI studies, obtaining much larger subject samples (e.g. n>40) is perhaps unrealistic. For such cases, it seems relevant to massively reduce the number of statistical tests. We have attempted to do so in chapters 4-6. Several much more sophisticated methods have also been developed, such as multivariate pattern recognition (Haynes & Rees, 2006), independent component analysis (Brown, Yamada, & Sejnowski, 2001), graph theory approaches (Bullmore & Sporns, 2009; Rubinov & Sporns, 2010) and cross-validated regression techniques (Wager, Atlas, Leotti, & Rilling, 2011). These methods are promising, since they reduce the number of statistical comparisons, and are generally better able to capture brain functioning (large-scale interconnections between regions) than the "standard" localized approaches. However, with the ever-increasing amount of novel analytic approaches, it is crucial that research results are independently replicated. For main effects of for example brain network properties, this has certainly been done (Zuo, Di Martino, et al., 2010a; Zuo, Kelly, et al., 2010b), but it is much less clear for interaction and between-subjects effects such as the typical group differences effects in clinical fMRI studies, as was argued in chapter 7.

Conclusion

Social anxiety and related personality traits are linked to alterations in structure and functioning of the neural emotion and motivation circuitries. Imbalances in these circuitries, towards more striatal punishment sensitivity and less cortical-subcortical communication, may form core neural-cognitive mechanisms in SAD. In addition, reward/punishment balance and emotion reactivity and regulation should be further integrated into models of social anxiety. The personality traits of neuroticism and extraversion may be differentially related to the regulation of the neural emotion and motivation circuitries. While neuroticism is linked to worry and sustained emotional dysregulation, extraversion is characterized by phasic increases in regulatory capacity and challenge appraisal of high demanding (stressful) situations. The combination of high neuroticism and low extraversion is therefore a predisposing factor for the development of SAD. Longitudinal research is needed to investigate individual trajectories, taking into account personality traits, the neural emotion and motivation circuitries, and the role each of these factors play in the vulnerability and resilience for developing, or recovery from, SAD. However, a representative insight into these and other neurobiological mechanisms related to psychiatric disorders cannot be obtained, as long as issues of statistical power and research bias are ignored. While the studies presented here may form

essential steps towards an understanding of the neurobiology of social anxiety, the importance of these results has to be evaluated by replication studies.

Chapter 9

References

Adelstein, J. S., Shehzad, Z., Mennes, M., Deyoung, C. G., Zuo, X.-N., Kelly, C., Margulies, D. S., et al. (2011). Personality Is Reflected in the Brain's Intrinsic Functional Architecture. *PLOS ONE*, *6*(11), e27633.

Affleck, G., & Tennen, H. (1996). Construing benefits from adversity: adaptational significance and dispositional underpinnings. *Journal of Personality*, *64*(4), 899–922.

American Psychiatric Association (1994). *Diagnostic & Statistical Manual for mental disorders (DSM)*. *4*th edn. American Psychiatric Press: Washington, DC.

Amodio, D. M., & Frith, C. D. (2006). Meeting of minds: the medial frontal cortex and social cognition. *Nature Reviews Neuroscience*, *7*(4), 268–277.

Amstadter, A. (2008). Emotion regulation and anxiety disorders. *Journal of Anxiety Disorders*, *22*(2), 211–221.

Arnsten, A. F. T. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Reviews Neuroscience*, *10*(6), 410–422.

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *NeuroImage*, *38*(1), 95–113.

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual review of neuroscience*, *28*, 403–450.

Baas, D., Aleman, A., & Kahn, R. S. (2004). Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. *Brain Research Reviews*, *45*(2), 96–103.

Bandettini, P. A. (2012). Twenty years of functional MRI: The science and the stories. *NeuroImage*, *62*(2), 575–588.

Baron, R. M., & Kenny, D. A. (1986). The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology*, *51*(6), 1173–1182.

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. *Nature Reviews Neuroscience*, *3*(7), 563–573.

Beck, A. T., Steer, R. A., & Carbin, M. G. (1988). Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. *Clinical Psychology Review*, *8*(1), 77–100.

Bennett, C. M., Wolford, G. L., & Miller, M. B. (2009). The principled control of false positives in neuroimaging. *Social Cognitive and Affective Neuroscience*, *4*(4), 417–422.

Berridge, K. C. (2004). Motivation concepts in behavioral neuroscience. *Physiology & Behavior*, *81*(2), 179–209.

Berridge, K. C. (2006). The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology*, *191*(3), 391–431.

Bickart, K. C., Wright, C. I., Dautoff, R. J., Dickerson, B. C., & Barrett, L. F. (2011). Amygdala volume and social network size in humans. *Nature Neuroscience*, *14*(2), 163–164.

Bienvenu, O. J., Brown, C., Samuels, J. F., Liang, K. Y., Costa, P. T., Eaton, W. W., & Nestadt, G. (2001). Normal personality traits and comorbidity among phobic, panic and major depressive disorders. *Psychiatry Research*, *102*(1), 73–85.

Bienvenu, O. J., Hettema, J. M., Neale, M. C., Prescott, C. A., & Kendler, K. S. (2007). Low extraversion and high neuroticism as indices of genetic and environmental risk for social phobia, agoraphobia, and animal phobia. *Am J Psychiatry*, *164*(11), 1714–1721.

Bienvenu, O. J., Samuels, J. F., Costa, P. T., Reti, I. M., Eaton, W. W., & Nestadt, G. (2004). Anxiety and depressive disorders and the five-factor model of personality: A higher- and lower-order personality trait investigation in a community sample. *Depression and Anxiety*, *20*(2), 92–97.

Bishop, S. J. (2007). Neurocognitive mechanisms of anxiety: an integrative account. *Trends in Cognitive Sciences*, *11*(7), 307–316.

Bishop, S. J. (2008). Trait anxiety and impoverished prefrontal control of attention. *Nature Neuroscience*, *12*(1), 92–98.

Blair, K. S., Geraci, M., Hollon, N., Otero, M., DeVido, J., Majestic, C., Jacobs, M., et al. (2010). Social Norm Processing in Adult Social Phobia: Atypically Increased Ventromedial Frontal Cortex Responsiveness to Unintentional (Embarrassing) Transgressions. *American Journal of Psychiatry*, *167*(12), 1526–1532.

Blair, K., Geraci, M., Devido, J., McCaffrey, D., Chen, G., Vythilingam, M., Ng, P., et al. (2008). Neural response to self- and other referential praise and criticism in generalized social phobia. *Archives of general psychiatry*, *65*(10), 1176–1184.

Blankstein, U., Chen, J. Y. W., Mincic, A. M., McGrath, P. A., & Davis, K. D. (2009). The complex minds of teenagers: Neuroanatomy of personality differs between sexes. *Neuropsychologia*, *47*(2), 599–603.

Blöte, A. W., Kint, M. J. W., Miers, A. C., & Westenberg, P. M. (2009). The relation between public speaking anxiety and social anxiety: a review. *Journal of Anxiety Disorders*, *23*(3), 305–313.

Borgeat, F., Stankovic, M., Khazaal, Y., Rouget, B. W., Baumann, M.-C., Riquier, F., O'Connor, K., et al. (2009). Does the Form or the Amount of Exposure Make a Difference in the Cognitive-Behavioral Therapy Treatment of Social Phobia? *The Journal of nervous and mental disease*, *197*(7), 507–513.

Borsboom, D., Cramer, A. O. J., Schmittmann, V. D., Epskamp, S., & Waldorp, L. J. (2011). The Small World of Psychopathology. *PLOS ONE*, *6*(11), e27407.

Bouchard, T. J., & Loehlin, J. C. (2001). Genes, evolution, and personality. *Behavior genetics*, *31*(3), 243–273.

Boureau, Y.-L., & Dayan, P. (2011). Opponency revisited: competition and cooperation between dopamine and serotonin. *Neuropsychopharmacology*, *36*(1), 74–97.

Bögels, S. M., Alden, L., Beidel, D. C., Clark, L. A., Pine, D. S., Stein, M. B., & Voncken, M. (2010). Social anxiety disorder: questions and answers for the DSM-V. *Depression and Anxiety*, *27*(2), 168–189.

Braver, T. S., Cole, M. W., & Yarkoni, T. (2010). Vive les differences! Individual variation in neural mechanisms of executive control. *Current Opinion in Neurobiology*, *20*(2), 242–250.

Bressler, S. L., & Menon, V. (2010). Large-scale brain networks in cognition: emerging methods and principles. *Trends in Cognitive Sciences*, *14*(6), 277–290.

Britton, J. C., Ho, S.-H., Taylor, S. F., & Liberzon, I. (2007). Neuroticism associated with neural activation patterns to positive stimuli. *Psychiatry Research: Neuroimaging*, *156*(3), 263–267.

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron*, *68*(5), 815–834.

Brown, G. D., Yamada, S., & Sejnowski, T. J. (2001). Independent component analysis at the neural cocktail party. *Trends in Neurosciences*, *24*(1), 54–63.

Bullmore, E., & Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature Reviews Neuroscience*, *10*(3), 186–198.

Bullmore, E., Fletcher, P., & Jones, P. B. (2009). Why psychiatry can't afford to be neurophobic. *The British journal of psychiatry : the journal of mental science*, *194*(4), 293–295.

Caceres, A., Hall, D. L., Zelaya, F. O., Williams, S. C. R., & Mehta, M. A. (2009). Measuring fMRI reliability with the intra-class correlation coefficient. *NeuroImage*, *45*(3), 758–768.

Campbell-Sills, L., Cohan, S. L., & Stein, M. B. (2006). Relationship of resilience to personality, coping, and psychiatric symptoms in young adults. *Behaviour Research and Therapy*, *44*(4), 585–599.

Canli, T. (2004). Functional brain mapping of extraversion and neuroticism: learning from individual differences in emotion processing. *Journal of Personality*, *72*(6), 1105–1132.

Canli, T., Sivers, H., Whitfield, S. L., Gotlib, I. H., & Gabrieli, J. D. E. (2002). Amygdala response to happy faces as a function of extraversion. *Science*, *296*(5576), 2191.

Canli, T., Zhao, Z., Desmond, J. E., Kang, E., Gross, J., & Gabrieli, J. D. E. (2001). An fMRI study of personality influences on brain reactivity to emotional stimuli. *Behavioral Neuroscience*, *115*(1), 33–42.

Carp, J. (2012). The secret lives of experiments: Methods reporting in the fMRI literature. *NeuroImage*, *63*(1), 289–300.

Carter, R. M., Macinnes, J. J., Huettel, S. A., & Adcock, R. A. (2009). Activation in the VTA and nucleus accumbens increases in anticipation of both gains and losses. *Frontiers in Behavioral Neuroscience*, *3*, 21.

Carver, C. S., & Connor-Smith, J. (2010). Personality and coping. *Annual Review of Psychology*, *61*, 679–704.

Carver, C., & White, T. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology*, *67*(2), 319–333.

Casey, B. J., Ruberry, E. J., Libby, V., Glatt, C. E., Hare, T., Soliman, F., Duhoux, S., et al. (2011). Transitional and translational studies of risk for anxiety. *Depression and Anxiety*, *28*(1), 18–28.

Chang, C., & Glover, G. H. (2009). Time–frequency dynamics of resting-state brain connectivity measured with fMRI. *NeuroImage*, *50*(1), 81–98.

Chorpita, B. F., & Barlow, D. H. (1998). The development of anxiety: The role of control in the early environment. *Psychological Bulletin*, *124*(1), 3–21.

Clark, D. M., & McManus, F. (2002). Information processing in social phobia. *Biological Psychiatry*, *51*(1), 92–100.

Clark, L., Watson, D., & Mineka, S. (1994). Temperament, personality, and the mood and anxiety disorders. *Journal of Abnormal Psychology*, *103*, 103–116.

Cloninger, C. (1986). A unified biosocial theory of personality and its role in the development of anxiety states. *Psychiatr Dev*, *4*(3), 167–226.

Cohen, J. (1962). The statistical power of abnormal-social psychological research: A review. *The Journal of Abnormal and Social Psychology*, *65*(3), 145–153. Journal of Abnormal and Social Psychology.

Cohen, J. (1990). Things I have learned (so far). *American Psychologist*, *45*(12), 1304–1312.

Cohen, J. (1992). A power primer. *Psychological Bulletin*, *112*(1), 155–159.

Cohen, J. (1994). The earth is round ($p < .05$). *American Psychologist*, *49*(12), 997–1003.

Cole, D. M., Smith, S. M., & Beckmann, C. F. (2010). Advances and pitfalls in the analysis and interpretation of resting-state FMRI data. *Frontiers in Systems Neuroscience*, *4*, 8.

Cools, R., Nakamura, K., & Daw, N. D. (2011). Serotonin and dopamine: unifying affective, activational, and decision functions. *Neuropsychopharmacology*, *36*(1), 98–113.

Costa, P. T., & McCrea, R. R. (1992). *Revised Neo Personality Inventory (Neo Pi-R) and Neo Five-Factor Inventory (Neo-Ffi)*. Odessa, FL: Psychological Assessment Resources.

Costafreda, S. G., Brammer, M. J., David, A. S., & Fu, C. H. Y. (2008). Predictors of amygdala activation during the processing of emotional stimuli: A meta-analysis of 385 PET and fMRI studies. *Brain Research Reviews*, *58*(1), 57–70.

Cramer, A. O. J., Waldorp, L. J., van der Maas, H. L. J., & Borsboom, D. (2010). Comorbidity: A network perspective. *The Behavioral and brain sciences*, *33*(2-3), 137–150.

Cremers, H. R., Demenescu, L. R., Aleman, A., Renken, R., van Tol, M.-J., van der Wee, N. J. A., Veltman, D. J., et al. (2010). Neuroticism modulates amygdala-prefrontal connectivity in response to negative emotional facial expressions. *NeuroImage*, *49*(1), 963–970.

Cribben, I., Haraldsdottir, R., Atlas, L. Y., Wager, T. D., & Lindquist, M. A. (2012). Dynamic connectivity regression: Determining state-related changes in brain connectivity. *NeuroImage*, *61*(4), 907–920.

Cumming, G. (2008). Replication and pIntervals: pValues Predict the Future Only Vaguely, but Confidence Intervals Do Much Better. *Perspectives on Psychological Science*, *3*(4), 286–300.

Damasio, A. R. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *351*(1346), 1413–1420.

Damasio, H., Grabowski, T., Frank, R., Galaburda, A., & Damasio, A. (1994). The return of Phineas Gage: clues about the brain from the skull of a famous patient. *Science*, *264*(5162), 1102–1105.

Davidson, R. J. (2002). Anxiety and affective style: role of prefrontal cortex and amygdala. *Biological Psychiatry*, *51*(1), 68–80.

Davidson, R. J., & McEwen, B. S. (2012). Social influences on neuroplasticity: stress and interventions to promote well-being. *Nature Neuroscience*, *15*(5), 689–695.

Davidson, R. J., Marshall, J. R., Tomarken, A. J., & Henriques, J. B. (2000). While a phobic waits: regional brain electrical and autonomic activity in social phobics during anticipation of public speaking. *Biological Psychiatry*, *47*(2), 85–95.

Davidson, R., & Irwin, W. (1999). The functional neuroanatomy of emotion and affective style. *Trends in Cognitive Sciences*, *3*(1), 11–21.

Davis, M., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Molecular Psychiatry*, *6*(1), 13–34.

De Angelis, C., Drazen, J. M., Frizelle, F. A., Haug, C., Hoey, J., Horton, R., Kotzin, S., et al. (2004). Clinical trial registration: a statement from the International Committee of Medical Journal Editors. *The New England journal of medicine*, *351*(12), 1250–1251.

Degnan, K. A., & Fox, N. A. (2007). Behavioral inhibition and anxiety disorders: multiple levels of a resilience process. *Development and psychopathology*, *19*(3), 729–746.

Delgado, M. R., Li, J., Schiller, D., & Phelps, E. A. (2008a). The role of the striatum in aversive learning and aversive prediction errors. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1511), 3787–3800.

Delgado, M. R., Nearing, K. I., LeDoux, J. E., & Phelps, E. A. (2008b). Neural Circuitry Underlying the Regulation of Conditioned Fear and Its Relation to Extinction. *Neuron*, *59*(5), 829–838.

Desmond, J. E., & Glover, G. H. (2002). Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses. *Journal of Neuroscience Methods*, *118*(2), 115–128.

Deyoung, C. G. (2010). Personality Neuroscience and the Biology of Traits. *Social and Personality Psychology Compass*, *4*(12), 1165–1180.

DeYoung, C. G., Hirsh, J. B., Shane, M. S., Papademetris, X., Rajeevan, N., & Gray, J. R. (2010). Testing Predictions From Personality Neuroscience: Brain Structure and the Big Five. *Psychological science*, *21*(6), 820–828.

Diekhof, E. K., Geier, K., Falkai, P., & Gruber, O. (2011). Fear is only as deep as the mind allows: a coordinate-based meta-analysis of neuroimaging studies on the regulation of negative affect. *NeuroImage*, *58*(1), 275–285.

Ding, J., Chen, H., Qiu, C., Liao, W., Warwick, J. M., Duan, X., Zhang, W., et al. (2011). Disrupted functional connectivity in social anxiety disorder: a resting-state fMRI study. *Magnetic Resonance Imaging*, *29*(5), 701–711.

Drevets, W. C., Price, J. L., & Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Structure and Function*, *213*(1-2), 93–118.

Duncan, L. E., & Keller, M. C. (2011). A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *American Journal of Psychiatry*, *168*(10), 1041–1049.

Duzel, E., Bunzeck, N., Guitart-Masip, M., Wittmann, B., Schott, B. H., & Tobler, P. N. (2009). Functional imaging of the human dopaminergic midbrain. *Trends in Neurosciences*, *32*(6), 321–328.

Eisenberger, N. I., Lieberman, M. D., & Satpute, A. B. (2005). Personality from a controlled processing perspective: an fMRI study of neuroticism, extraversion, and self-consciousness. *Cognitive, affective & behavioral neuroscience*, *5*(2), 169–181.

Elzinga, B. M., Spinhoven, P., Berretty, E., de Jong, P., & Roelofs, K. (2010). The role of childhood abuse in HPA-axis reactivity in Social Anxiety Disorder: a pilot study. *Biological Psychology*, *83*(1), 1–6.

Eskildsen, A., Hougaard, E., & Rosenberg, N. K. (2010). Pre-treatment patient variables as predictors of drop-out and treatment outcome in cognitive behavioural therapy for social phobia: A systematic review. *Nordic journal of psychiatry*, *64*(2), 94–105.

Etkin, A., & Wager, T. (2007). Functional Neuroimaging of Anxiety: A Meta-Analysis of Emotional Processing in PTSD, Social Anxiety Disorder, and Specific Phobia. *Am J Psychiatry*, *167*, 1–13.

Etkin, A., Egner, T., Peraza, D. M., Kandel, E. R., & Hirsch, J. (2006). Resolving Emotional Conflict: A Role for the Rostral Anterior Cingulate Cortex in Modulating Activity in the Amygdala. *Neuron*, *51*(6), 871–882.

Etkin, A., Prater, K. E., Schatzberg, A. F., Menon, V., & Greicius, M. D. (2009). Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Archives of general psychiatry*, *66*(12), 1361–1372.

Eysenck, H. (1967). *The biological basis of personality*. Springfield, IL: Thomas.

Falk, R., & Greenbaum, C. W. (1995). Significance Tests Die Hard: The Amazing Persistence of a Probabilistic Misconception. *Theory & Psychology*, *5*(1), 75–98.

Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin & Review*, *1*(4), 429–438.

Faria, V., Appel, L., Åhs, F., Linnman, C., Pissiota, A., Frans, O., Bani, M., et al. (2012). Amygdala Subregions Tied to SSRI and Placebo Response in Patients with Social Anxiety Disorder. *Neuropsychopharmacology*.

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods*, *39*(2), 175–191.

Feder, A., Nestler, E. J., & Charney, D. S. (2009). Psychobiology and molecular genetics of resilience. *Nature Reviews Neuroscience*, *10*(6), 446–457.

Fedoroff, I. C., & Taylor, S. (2001). Psychological and Pharmacological Treatments of Social Phobia: A Meta-Analysis. *Journal of clinical psychopharmacology*, *21*(3), 311.

Feldman, P. J., Cohen, S., Hamrick, N., & Lepore, S. J. (2004). Psychological stress, appraisal, emotion and Cardiovascular response in a public speaking task. *Psychology & Health*, *19*(3), 353–368.

Fitzgerald, D. A., Angstadt, M., Jelsone, L. M., Nathan, P. J., & Phan, K. L. (2006). Beyond threat: Amygdala reactivity across multiple expressions of facial affect. *NeuroImage*, *30*(4), 1441–1448.

Fliessbach, K., Rohe, T., Linder, N. S., Trautner, P., Elger, C. E., & Weber, B. (2010). Retest reliability of reward-related BOLD signals. *NeuroImage*, *50*(3), 1168–1176.

Freitas-Ferrari, M. C., Hallak, J. E. C., Trzesniak, C., Filho, A. S., Machado-de-Sousa, J. P., Chagas, M. H. N., Nardi, A. E., et al. (2010). Neuroimaging in social anxiety disorder: a systematic review of the literature. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(4), 565–580.

Fresco, D. M., Coles, M. E., Heimberg, R. G., Liebowitz, M. R., Hami, S., Stein, M. B., & Goetz, D. (2001). The Liebowitz Social Anxiety Scale: a comparison of the psychometric properties of self-report and clinician-administered formats. *Psychological medicine*, *31*(6), 1025–1035.

Friston, K. J. (2011). Functional and Effective Connectivity: A Review. *Brain Connectivity*, *1*(1), 13–36

Friston, K. J., Buechel, C., Fink, G. R., Morris, J., Rolls, E., & Dolan, R. J. (1997). Psychophysiological and modulatory interactions in neuroimaging. *NeuroImage*, *6*(3), 218–229.

Friston, K. J., Holmes, A. P., Worsley, K. J., Poline, J. P., Frith, C. D., & Frackowiak, R. S. J. (2004). Statistical parametric maps in functional imaging: A general linear approach. *Human Brain Mapping*, *2*(4), 189–210.

Furmark, T., Appel, L., Michelgard, a., Wahlstedt, k., Ahs, f., Zancan, s., Jacobsson, E., et al. (2005). Cerebral Blood Flow Changes After Treatment of Social Phobia with the Neurokinin-1 Antagonist GR205171, Citalopram, or Placebo. *Biological Psychiatry*, *58*(2), 132–142.

Gaston, J. E., Abbott, M. J., Rapee, R. M., & Neary, S. A. (2006). Do empirically supported treatments generalize to private practice? A benchmark study of a cognitive-behavioural group treatment programme for social phobia. *The British journal of clinical psychology / the British Psychological Society*, *45*(1), 33–48.

Gelernter, C. S., Uhde, T. W., Cimbolic, P., Arnkoff, D. B., Vittone, B. J., Tancer, M. E., & Bartko, J. J. (1991). Cognitive-behavioral and pharmacological treatments of social phobia. A controlled study. *Archives of general psychiatry*, *48*(10), 938–945.

Gelman, A., & Weakliem, D. (2009). Of beauty, sex and power. *American Scientist*, *97*(4), 310–316.

Ghashghaei, H. T., Hilgetag, C. C., & Barbas, H. (2007). Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *NeuroImage*, *34*(3), 905–923.

Gigerenzer, G. (2004). Mindless statistics. *The Journal of Socio-Economics*, *33*(5), 587–606.

Goldberg, D. (2000). Plato versus Aristotle: Categorical and dimensional models for common mental disorders. *Comprehensive psychiatry*, *41*(2), 8–13.

Goldin, P. R., & Gross, J. J. (2010). Effects of mindfulness-based stress reduction (MBSR) on emotion regulation in social anxiety disorder. *Emotion*, *10*(1), 83–91.

Goldin, P. R., Manber, T., Hakimi, S., Canli, T., & Gross, J. J. (2009a). Neural bases of social anxiety disorder: emotional reactivity and cognitive regulation during social and physical threat. *Archives of general psychiatry*, *66*(2), 170–180.

Goldin, P. R., Manber-Ball, T., Werner, K., Heimberg, R., & Gross, J. J. (2009b). Neural Mechanisms of Cognitive Reappraisal of Negative Self-Beliefs in Social Anxiety Disorder. *Biological Psychiatry*, 66(12), 1091-1099

Goldin, P., Ziv, M., Jazaieri, H., Hahn, K., & Gross, J. J. (2012). MBSR vs aerobic exercise in social anxiety: fMRI of emotion regulation of negative self-beliefs. *Social Cognitive and Affective Neuroscience*.

Gramer, M., & Saria, K. (2007). Effects of social anxiety and evaluative threat on cardiovascular responses to active performance situations. *Biological Psychology*, *74*(1), 67–74.

Gray, J. A. (1978). The neuropsychology of anxiety. *British Journal of Psychology*, *69*(4), 417–434. Wiley Online Library.

Gross, J. J. (2010). The Future's So Bright, I Gotta Wear Shades. *Emotion review*, *2*(3), 212–216.

Gross, J. J., & Barrett, L. F. (2011). Emotion Generation and Emotion Regulation: One or Two Depends on Your Point of View. *Emotion review*, *3*(1), 8–16.

Guyer, A. E., Choate, V. R., Detloff, A., Benson, B., Nelson, E. E., Perez-Edgar, K., Fox, N. A., et al. (2012). Striatal Functional Alteration During Incentive Anticipation in Pediatric Anxiety Disorders. *Am J Psychiatry*, *169*(2), 205–212.

Guyer, A. E., Lau, J. Y. F., McClure-Tone, E. B., Parrish, J., Shiffrin, N. D., Reynolds, R. C., Chen, G., et al. (2008). Amygdala and ventrolateral prefrontal cortex function during anticipated peer evaluation in pediatric social anxiety. *Archives of general psychiatry*, *65*(11), 1303–1312.

Guyer, A. E., Nelson, E. E., Perez-Edgar, K., Hardin, M. G., Roberson-Nay, R., Monk, C. S., Bjork, J. M., et al. (2006). Striatal functional alteration in adolescents characterized by early childhood behavioral inhibition. *Journal of Neuroscience*, *26*(24), 6399–6405.

Haas, B. W., Constable, R. T., & Canli, T. (2008). Stop the sadness: Neuroticism is associated with sustained medial prefrontal cortex response to emotional facial expressions. *NeuroImage*, *42*(1), 385–392.

Haas, B. W., Omura, K., Amin, Z., Constable, R. T., & Canli, T. (2006). Functional connectivity with the anterior cingulate is associated with extraversion during the emotional Stroop task. *Social neuroscience*, *1*(1), 16–24.

Haas, B. W., Omura, K., Constable, R. T., & Canli, T. (2007). Emotional conflict and neuroticism: Personality-dependent activation in the amygdala and subgenual anterior cingulate. *Behavioral Neuroscience*, *121*(2), 249–256.

Haber, S. N., & Knutson, B. (2010). The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*, *35*(1), 4-26.

Hahn, A., Stein, P., Windischberger, C., Weissenbacher, A., Spindelegger, C., Moser, E., Kasper, S., et al. (2011). Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. *NeuroImage*, *56*(3), 881–889.

Hamaker, E. L., Nesselroade, J. R., & Molenaar, P. C. M. (2007). The integrated trait–state model. *Journal of research in personality*, *41*(2), 295–315.

Hamann, S. (2004). Individual differences in emotion processing. *Current Opinion in Neurobiology*, *14*(2), 233–238.

Hardin, M. G., Perez-Edgar, K., Guyer, A. E., Pine, D. S., Fox, N. A., & Ernst, M. (2006). Reward and punishment sensitivity in shy and non-shy adults: Relations between social and motivated behavior. *Personality and Individual Differences*, *40*(4), 699–711.

Hartley, C. A., & Phelps, E. A. (2009). Changing Fear: The Neurocircuitry of Emotion Regulation. *Neuropsychopharmacology*, *35*(1), 136–146.

Haslam, N. (2003). Categorical versus dimensional models of mental disorder: The taxometric evidence. *Australian and New Zealand Journal of Psychiatry*. 37(6), 696-704

Hastings, R. S., Parsey, R. V., Oquendo, M. A., Arango, V., & Mann, J. J. (2004). Volumetric analysis of the prefrontal cortex, amygdala, and hippocampus in major depression. *Neuropsychopharmacology*, *29*(5), 952–959.

Haynes, J.-D., & Rees, G. (2006). Decoding mental states from brain activity in humans. *Nature Reviews Neuroscience*, *7*(7), 523–534.

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological Psychiatry*, *49*(12), 1023–1039.

Heimberg, R. G., Liebowitz, M. R., Hope, D. A., Schneier, F. R., Holt, C. S., Welkowitz, L. A., Juster, H. R., et al. (1998). Cognitive behavioral group therapy vs phenelzine therapy for social phobia: 12-week outcome. *Archives of general psychiatry*, *55*(12), 1133–1141.

Heinrichs, N., & Hofmann, S. G. (2001). Information processing in social phobia: a critical review. *Clinical Psychology Review*, *21*(5), 751–770.

Henckens, M. J. A. G., van Wingen, G. A., Joëls, M., & Fernández, G. (2010). Time-dependent effects of corticosteroids on human amygdala processing. *Journal of Neuroscience*, *30*(38), 12725–12732.

Henckens, M. J. A. G., van Wingen, G. A., Joëls, M., & Fernández, G. (2011). Corticosteroid Induced Decoupling of the Amygdala in Men. *Cerebral Cortex*.

Henningsen, P., & Kirmayer, L. J. (2000). Mind beyond the net: implications of cognitive neuroscience for cultural psychiatry. *Transcultural Psychiatry*, *37*(4), 467–494.

Hofmann, S. G. (2005). Perception of control over anxiety mediates the relation between catastrophic thinking and social anxiety in social phobia. *Behaviour Research and Therapy*, *43*(7), 885–895.

Hofmann, S. G. (2007). Cognitive factors that maintain social anxiety disorder: a comprehensive model and its treatment implications. *Cognitive behaviour therapy*, *36*(4), 193–209.

Hofmann, S. G. (2010). Recent advances in the psychosocial treatment of social anxiety disorder. *Depression and Anxiety*, *27*(12), 1073–1076.

Holtforth, M. G. (2008). Avoidance motivation in psychological problems and psychotherapy. *Psychotherapy research : journal of the Society for Psychotherapy Research*, *18*(2), 147–159.

Hughes, B. M. (2007). Social support in ordinary life and laboratory measures of cardiovascular reactivity: gender differences in habituation-sensitization. *Annals of behavioral medicine : a publication of the Society of Behavioral Medicine*, *34*(2), 166–176.

Iacovella, V., & Hasson, U. (2011). The relationship between BOLD signal and autonomic nervous system functions: implications for processing of "physiological noise." *Magnetic Resonance Imaging*, *29*(10), 1338–1345.

Ioannidis, J. P. A. (2005). Why Most Published Research Findings Are False. *PLOS Medicine*, *2*(8), e124.

Ioannidis, J. P. A. (2008). Why Most Discovered True Associations Are Inflated. *Epidemiology*, *19*(5), 640–648.

Ioannidis, J. P. A. (2011). Excess Significance Bias in the Literature on Brain Volume Abnormalities. *Archives of general psychiatry*, *68*(8), 773–780.

Irle, E. (2010). Reduced amygdalar and hippocampal size in adults with generalized social phobia. *Journal of psychiatry & neuroscience : JPN*, *35*(2), 126–131. Canadian Medical Association.

Jackson, J., Balota, D. A., & Head, D. (2009). Exploring the relationship between personality and regional brain volume in healthy aging. *Neurobiology of aging*.

Joëls, M., & Baram, T. Z. (2009). The neuro-symphony of stress. *Nature Reviews Neuroscience*, *10*(6), 459–466.

Johansen-Berg, H., Gutman, D. A., Behrens, T. E. J., Matthews, P. M., Rushworth, M. F. S., Katz, E., Lozano, A. M., et al. (2008). Anatomical Connectivity of the Subgenual Cingulate Region Targeted with Deep Brain Stimulation for Treatment-Resistant Depression. *Cerebral Cortex*, *18*(6), 1374–1383.

Jylhä, P., & Isometsä, E. (2006). The relationship of neuroticism and extraversion to symptoms of anxiety and depression in the general population. *Depression and Anxiety*, *23*(5), 281–289.

Kahneman, D., & Tversky, A. (1984). Choices, values, and frames. *American Psychologist*, *39*(4), 341.

Kapur, S., Phillips, A. G., & Insel, T. R. (2012). Why has it taken so long for biological psychiatry to develop clinical tests and what to do about it? *Molecular Psychiatry*, 17(12), 1174-1179.

Kaufman, J., Plotsky, P. M., Nemeroff, C. B., & Charney, D. S. (2000). Effects of early adverse experiences on brain structure and function: clinical implications. *Biological Psychiatry*, *48*(8), 778–790.

Keightley, M. L., Seminowicz, D. A., Bagby, R. M., Costa, P. T., Fossati, P., & Mayberg, H. S. (2003). Personality influences limbic-cortical interactions during sad mood induction. *NeuroImage*, *20*(4), 2031–2039.

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of general psychiatry*, *62*(6), 617–627.

Kienast, T., Hariri, A. R., Schlagenhauf, F., Wrase, J., Sterzer, P., Buchholz, H. G., Smolka, M. N., et al. (2008). Dopamine in amygdala gates limbic processing of aversive stimuli in humans. *Nature Neuroscience*, *11*(12), 1381–1382.

Kim, M. J., Loucks, R. A., Palmer, A. L., Brown, A. C., Solomon, K. M., Marchante, A. N., & Whalen, P. J. (2011). The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. *Behavioural Brain Research*, *223*(2), 403–410.

Kimbrel, N. A. (2008). A model of the development and maintenance of generalized social phobia. *Clinical Psychology Review*, *28*(4), 592–612.

Kimbrel, N. A., Mitchell, J. T., & Nelson-Gray, R. O. (2010). An examination of the relationship between behavioral approach system (BAS) sensitivity and social interaction anxiety. *Journal of Anxiety Disorders*, *24*(3), 372–378.

Knutson, B., & Greer, S. M. (2008). Anticipatory affect: neural correlates and consequences for choice. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1511), 3771–3786.

Knutson, B., Fong, G. W., Adams, C. M., Varner, J. L., & Hommer, D. (2001). Dissociation of reward anticipation and outcome with event-related fMRI. *NeuroReport*, *12*(17), 3683–3687.

Kober, H., Barrett, L. F., Joseph, J., Bliss-Moreau, E., Lindquist, K., & Wager, T. D. (2008). Functional grouping and cortical–subcortical interactions in emotion: A meta-analysis of neuroimaging studies. *NeuroImage*, *42*(2), 998–1031.

Koob, G. F. (2008). A Role for Brain Stress Systems in Addiction. *Neuron*, *59*(1), 11–34.

Kotov, R., Gamez, W., Schmidt, F., & Watson, D. (2010). Linking "big" personality traits to anxiety, depressive, and substance use disorders: a meta-analysis. *Psychological Bulletin*, *136*(5), 768–821.

Kriegeskorte, N., Simmons, W. K., Bellgowan, P. S. F., & Baker, C. I. (2009). Circular analysis in systems neuroscience: the dangers of double dipping. *Nature Neuroscience*, *12*(5), 535–540.

Kringelbach, M. L., & Rolls, E. T. (2004). The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Progress in neurobiology*, *72*(5), 341–372.

Larsen, R. J., & Ketelaar, T. (1991). Personality and susceptibility to positive and negative emotional states. *Journal of Personality and Social Psychology*, *61*(1), 132–140.

Lecrubier, Y., Sheehan, D., Weiller, E., Amorim, P., Bonora, I., Harnett Sheehan, K., Janavs, J., et al. (1997). The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: reliability and validity according to the CIDI. *European Psychiatry*, *12*(5), 224–231.

LeDoux, J. (1998). *The emotional brain*. New York : Simon and Schuste.

LeDoux, J. E. (2000). Emotion Circuits in the Brain. *Annual review of neuroscience*, *23*(1), 155–184.

LeDoux, J. E. (2002). Synaptic Self. *Viking Pinguin*. New York.

Lee, H., Heller, A. S., van Reekum, C. M., Nelson, B., & Davidson, R. J. (2012). Amygdala-prefrontal coupling underlies individual differences in emotion regulation. *NeuroImage*, 62(3), 1575-1581.

Levita, L., Hoskin, R., & Champi, S. (2012). Avoidance of harm and anxiety: A role for the nucleus accumbens. *NeuroImage*, *62*(1), 189–198.

Lewis-Morrarty, E., Degnan, K. A., Chronis-Tuscano, A., Rubin, K. H., Cheah, C. S. L., Pine, D. S., Henderon, H. A., et al. (2012). Maternal over-control moderates the association between early childhood behavioral inhibition and adolescent social anxiety symptoms. *Journal of abnormal child psychology*, *40*(8), 1363–1373.

Li, Y., Qin, W., Jiang, T., Zhang, Y., & Yu, C. (2012). Sex-Dependent Correlations between the Personality Dimension of Harm Avoidance and the Resting-State Functional Connectivity of Amygdala Subregions. *PLOS ONE*, *7*(4), e35925.

Liao, W., Qiu, C., Gentili, C., Walter, M., Pan, Z., Ding, J., Zhang, W., et al. (2010). Altered effective connectivity network of the amygdala in social anxiety disorder: a resting-state FMRI study. *PLOS ONE*, *5*(12), e15238.

Lieberman, M. D., & Cunningham, W. A. (2009). Type I and Type II error concerns in fMRI research: re-balancing the scale. *Social Cognitive and Affective Neuroscience*, *4*(4), 423–428.

Lindquist, K. A., Wager, T. D., Kober, H., Bliss-Moreau, E., & Barrett, L. F. (2012). The brain basis of emotion: A meta-analytic review. The Behavioral and brain sciences, 35(3), 121–143.

Lindquist, M. A., Waugh, C., & Wager, T. D. (2007). Modeling state-related fMRI activity using change-point theory. *NeuroImage*, *35*(3), 1125–1141.

Liu, X. S. (2011). Implications of statistical power for confidence intervals. *British Journal of Mathematical and Statistical Psychology*, 65(3), 427-437

Loewenstein, G. F., Weber, E. U., Hsee, C. K., & Welch, N. (2001). Risk as feelings. *Psychological Bulletin*, *127*(2), 267–286.

Loftus, G. (1996). Psychology will be a much better science when we change the way we analyze data. *Current Directions in Psychological Science*, 161–171

Loftus, G. R. (1991). On the Tyranny of Hypothesis Testing in the Social Sciences. *Contemporary psychology*, *36*(2), 102–105.

Logothetis, N. K. (2008). What we can do and what we cannot do with fMRI. *Nature*, *453*(7197), 869–878.

Lorberbaum, J. P., Kose, S., Johnson, M. R., Arana, G. W., Sullivan, L. K., Hamner, M. B., Ballenger, J. C., et al. (2004). Neural correlates of speech anticipatory anxiety in generalized social phobia. *NeuroReport*, *15*(18), 2701–2705.

Lundqvist, D., Flykt, A., & Ohman, A. (1998). Karolinska Directed Emotional Faces. *Karolinska Institue, Stockholm*.

Maier, S. F., & Watkins, L. R. (2010). Role of the medial prefrontal cortex in coping and resilience. *Brain Research*, *1355*, 52–60.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*, *19*(3), 1233–1239.

Margulies, D. S., Böttger, J., Long, X., Lv, Y., Kelly, C., Schäfer, A., Goldhahn, D., et al. (2010). Resting developments: a review of fMRI post-processing methodologies for spontaneous brain activity. *Magnetic Resonance Materials in Physics, Biology and Medicine*, *23*(5-6), 289–307.

Markon, K. E., Krueger, R. F., & Watson, D. (2005). Delineating the structure of normal and abnormal personality: an integrative hierarchical approach. *Journal of Personality and Social Psychology*, *88*(1), 139.

Masten, A. S. (2001). Ordinary magic: Resilience processes in development. *American Psychologist*, *56*(3), 227–238.

Matsumoto, M., & Hikosaka, O. (2009). Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature*, *459*(7248), 837–841.

Maxwell, S. E. (2004). The Persistence of Underpowered Studies in Psychological Research: Causes, Consequences, and Remedies. *Psychological methods*, *9*(2), 147–163.

Mayberg, H. S., Lozano, A. M., Voon, V., McNeely, H. E., Seminowicz, D., Hamani, C., Schwalb, J. M., et al. (2005). Deep Brain Stimulation for Treatment-Resistant Depression. *Neuron*, *45*(5), 651–660.

McCrae, R. R., & Costa, P. T. (1991). Adding Liebe und Arbeit: The Full Five-Factor Model and Well-Being. *Personality and Social Psychology Bulletin*, *17*(2), 227–232.

Meehl, P. (1967). Theory-testing in psychology and physics: A methodological paradox. *Philosophy of science*, *34*(2), 103–115.

Meehl, P. E. (2006). Comorbidity and Taxometrics. *Clinical Psychology: Science and Practice*, *8*(4), 507–519.

Mennes, M., Kelly, C., Zuo, X.-N., Di Martino, A., Biswal, B. B., Castellanos, F. X., & Milham, M. P. (2010). Inter-individual differences in resting-state functional connectivity predict task-induced BOLD activity. *NeuroImage*, 50(4), 1690–1701.

Mennes, M., Zuo, X.-N., Kelly, C., Di Martino, A., Zang, Y.-F., Biswal, B., Castellanos, F. X., et al. (2011). Linking inter-individual differences in neural activation and behavior to intrinsic brain dynamics. *NeuroImage*, *54*(4), 2950–2959.

Menon, V. (2011). Large-scale brain networks and psychopathology: a unifying triple network model. *Trends in Cognitive Sciences*, *15*(10), 483–506.

Miers, A. C., Blöte, A. W., de Rooij, M., Bokhorst, C. L., & Westenberg, P. M. (2012). Trajectories of Social Anxiety during Adolescence and Relations with Cognition, Social Competence, and Temperament. *Journal of abnormal child psychology*, 41(1), 97–110.

Milad, M. R., & Rauch, S. L. (2007). The role of the orbitofrontal cortex in anxiety disorders. *Annals of the New York Academy of Sciences*, *1121*, 546–561.

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual review of neuroscience*, *24*, 167–202.

Miller, J. (2009). What is the probability of replicating a statistically significant effect? *Psychonomic Bulletin & Review*, *16*(4), 617–640.

Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: It's not what you thought it was. *American Psychologist*, *61*(1), 10–26.

Miskovic, V., Ashbaugh, A. R., Santesso, D. L., McCabe, R. E., Antony, M. M., & Schmidt, L. A. (2010). Frontal brain oscillations and social anxiety: a cross-frequency spectral analysis during baseline and speech anticipation. *Biological Psychology*, *83*(2), 125–132.

Miskovic, V., Moscovitch, D. A., Santesso, D. L., McCabe, R. E., Antony, M. M., & Schmidt, L. A. (2011). Changes in EEG cross-frequency coupling during cognitive behavioral therapy for social anxiety disorder. *Psychological science*, *22*(4), 507–516.

Mitra, R., Jadhav, S., McEwen, B. S., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(26), 9371–9376.

Mizrahi, R. (2010). Advances in PET analyses of stress and dopamine. *Neuropsychopharmacology*, *35*(1), 348–349.

Mobbs, D., Hagan, C. C., Azim, E., Menon, V., & Reiss, A. L. (2005). Personality predicts activity in reward and emotional regions associated with humor. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(45), 16502–16506.

Morgan, B. E., van Honk, J., Hermans, E. J., Scholten, M. R. M., Stein, D. J., & Kahn, R. S. (2009). Gray's BIS/BAS dimensions in non-comorbid, non-medicated social anxiety disorder. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, *10*(4 Pt 3), 925–928.

Moscovitch, D. A., & Hofmann, S. G. (2007). When ambiguity hurts: Social standards moderate self-appraisals in generalized social phobia. *Behaviour Research and Therapy*, *45*(5), 1039–1052.

Moser, J. S., Huppert, J. D., Foa, E. B., & Simons, R. F. (2012). Interpretation of ambiguous social scenarios in social phobia and depression: evidence from event-related brain potentials. *Biological Psychology*, *89*(2), 387–397.

Nachmias, M., Gunnar, M., Mangelsdorf, S., Parritz, R. H., & Buss, K. (1996). Behavioral inhibition and stress reactivity: the moderating role of attachment security. *Child development*, *67*(2), 508–522.

Naragon-Gainey, K., & Watson, D. (2011). Clarifying the dispositional basis of social anxiety: A hierarchical perspective. *Personality and Individual Differences*, *50*(7), 926–934.

Neal, J. A., & Edelmann, R. J. (2003). The etiology of social phobia: toward a developmental profile. *Clinical Psychology Review*, *23*(6), 761–786.

Nichols, T. E. (2012). Multiple testing corrections, nonparametric methods, and random field theory. *NeuroImage*, *62*(2), 811–815.

Nichols, T., & Hayasaka, S. (2003). Controlling the familywise error rate in functional neuroimaging: a comparative review. *Statistical Methods in Medical Research*, *12*(5), 419–446.

Nickerson, R. S. (2000). Null hypothesis significance testing: a review of an old and continuing controversy. *Psychological methods*, *5*(2), 241–301.

Nieuwenhuis, S., Forstmann, B. U., & Wagenmakers, E.-J. (2011). Erroneous analyses of interactions in neuroscience: a problem of significance. *Nature Neuroscience*, *14*(9), 1105–1107.

Nikolova, Y. S., Bogdan, R., Brigidi, B. D., & Hariri, A. R. (2012). Ventral Striatum Reactivity to Reward and Recent Life Stress Interact to Predict Positive Affect. *Biological Psychiatry*, *72*(2), 157–163.

Northoff, G., & Bermpohl, F. (2004). Cortical midline structures and the self. *Trends in Cognitive Sciences*, *8*(3), 102–107.

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences*, *9*(5), 242–249.

Ode, S., Hilmert, C. J., Zielke, D. J., & Robinson, M. D. (2010). Neuroticism's importance in understanding the daily life correlates of heart rate variability. *Emotion*, *10*(4), 536–543.

Oei, N. Y. L., Veer, I. M., Wolf, O. T., Spinhoven, P., Rombouts, S. A. R. B., & Elzinga, B. M. (2012). Stress shifts brain activation towards ventral "affective" areas during emotional distraction. *Social Cognitive and Affective Neuroscience*, *7*(4), 403–412.

Olff, M., Langeland, W., & Gersons, B. P. R. (2005). Effects of appraisal and coping on the neuroendocrine response to extreme stress. *Neuroscience & Biobehavioral Reviews*, *29*(3), 457–467.

Omura, K., Todd Constable, R., & Canli, T. (2005). Amygdala gray matter concentration is associated with extraversion and neuroticism. *NeuroReport*, *16*(17), 1905–1908.

Ormel, J., Rosmalen, J., & Farmer, A. (2004). Neuroticism: a non-informative marker of vulnerability to psychopathology. *Social Psychiatry and Psychiatric Epidemiology*, *39*(11), 906–912.

Ossewaarde, L., Qin, S., van Marle, H. J. F., van Wingen, G. A., Fernández, G., & Hermans, E. J. (2011). Stress-induced reduction in reward-related prefrontal cortex function. *NeuroImage*, *55*(1), 345–352.

Oswald, L. M., Zandi, P., Nestadt, G., Potash, J. B., Kalaydjian, A. E., & Wand, G. S. (2006). Relationship between Cortisol Responses to Stress and Personality. *Neuropsychopharmacology*, *31*(7), 1583–1591.

Passamonti, L., Rowe, J. B., Ewbank, M., Hampshire, A., Keane, J., & Calder, A. J. (2008). Connectivity from the ventral anterior cingulate to the amygdala is modulated by appetitive motivation in response to facial signals of aggression. *NeuroImage*, *43*(3), 562–570.

Penninx, B. W. J. H., Beekman, A. T. F., Smit, J. H., Zitman, F. G., Nolen, W. A., Spinhoven, P., Cuijpers, P., et al. (2008). The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. *International Journal of Methods in Psychiatric Research*, *17*(3), 121–140.

Perlis, R. H. (2011). Translating biomarkers to clinical practice. *Molecular Psychiatry*, *16*(11), 1076–1087.

Pessoa, L. (2008). On the relationship between emotion and cognition. *Nature Reviews Neuroscience*, *9*(2), 148–158.

Pessoa, L., & Adolphs, R. (2010). Emotion processing and the amygdala: from a 'low road' to "many roads" of evaluating biological significance. *Nature Reviews Neuroscience*, *11*(11), 773–783.

Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., Egan, M. F., et al. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience*, *8*(6), 828–834.

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional Neuroanatomy of Emotion: A Meta-Analysis of Emotion Activation Studies in PET and fMRI. *NeuroImage*, *16*(2), 331–348.

Piccinelli, M., & Wilkinson, G. (2000). Gender differences in depression. Critical review. *The British journal of psychiatry : the journal of mental science*, *177*, 486–492.

Plichta, M. M., Schwarz, A. J., Grimm, O., Morgen, K., Mier, D., Haddad, L., Gerdes, A. B. M., et al. (2012). Test-retest reliability of evoked BOLD signals from a cognitive-emotive fMRI test battery. *NeuroImage*, *60*(3), 1746–1758.

Poldrack, R. A. (2006). Region of interest analysis for fMRI. *Social Cognitive and Affective Neuroscience*, *2*(1), 67–70.

Poldrack, R. A. (2011). The future of fMRI in cognitive neuroscience. *NeuroImage*, 62(2), 1216–1220.

Pollet, T. V., Roberts, S. G. B., & Dunbar, R. I. M. (2011). Extraverts Have Larger Social Network Layers. *Journal of Individual Differences*, *32*(3), 161–169.

Price, J. L. (2003). Comparative aspects of amygdala connectivity. *Annals of the New York Academy of Sciences*, *985*, 50–58.

Price, J. L., & Drevets, W. C. (2010). Neurocircuitry of mood disorders. *Neuropsychopharmacology*, *35*(1), 192–216.

Pruessner, J. C., Champagne, F., Meaney, M. J., & Dagher, A. (2004). Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. *Journal of Neuroscience*, *24*(11), 2825–2831.

Pruessner, J. C., Dedovic, K., Khalili-Mahani, N., Engert, V., Pruessner, M., Buss, C., Renwick, R., et al. (2008). Deactivation of the Limbic System During Acute Psychosocial Stress: Evidence from Positron Emission Tomography and Functional Magnetic Resonance Imaging Studies. *Biological Psychiatry*, *63*(2), 234–240.

Pruessner, J. C., Gaab, J., Hellhammer, D. H., Lintz, D., Schommer, N., & Kirschbaum, C. (1997). Increasing correlations between personality traits and cortisol stress responses obtained by data aggregation. *Psychoneuroendocrinology*, *22*(8), 615–625.

Putman, P., Antypa, N., Crysovergi, P., & van der Does, W. A. J. (2010). Exogenous cortisol acutely influences motivated decision making in healthy young men. *Psychopharmacology*, *208*(2), 257–263.

Qiu, C., Liao, W., Ding, J., Feng, Y., Zhu, C., Nie, X., Zhang, W., et al. (2011). Regional homogeneity changes in social anxiety disorder: a resting-state fMRI study. *Psychiatry Research*, *194*(1), 47–53.

Quirk, G. J., & Beer, J. S. (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Current Opinion in Neurobiology*, *16*(6), 723–727.

Quirk, G. J., & Mueller, D. (2007). Neural Mechanisms of Extinction Learning and Retrieval. *Neuropsychopharmacology*, *33*(1), 56–72.

Raichle, M. E. (2010). Two views of brain function. *Trends in Cognitive Sciences*, *14*(4), 180–190.

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(2), 676.

Rapee, R. M., & Heimberg, R. G. (1997). A cognitive-behavioral model of anxiety in social phobia. *Behaviour Research and Therapy*, *35*(8), 741–756.

Rapee, R. M., & Spence, S. H. (2004). The etiology of social phobia: empirical evidence and an initial model. *Clinical Psychology Review*, *24*(7), 737–767.

Rapee, R. M., Gaston, J. E., & Abbott, M. J. (2009). Testing the efficacy of theoretically derived improvements in the treatment of social phobia. *Journal of consulting and clinical psychology*, *77*(2), 317–327.

Rauch, S. L., Milad, M. R., Orr, S. P., Quinn, B. T., Fischl, B., & Pitman, R. K. (2005). Orbitofrontal thickness, retention of fear extinction, and extraversion. *NeuroReport*, *16*(17), 1909–1912.

Reuter, M., Stark, R., Hennig, J., Walter, B., Kirsch, P., Schienle, A., & Vaitl, D. (2004). Personality and Emotion: Test of Gray's Personality Theory by Means of an fMRI Study. *Behavioral Neuroscience*, *118*(3), 462–469.

Robinson, M. D., Ode, S., Moeller, S. K., & Goetz, P. W. (2007a). Neuroticism and Affective Priming: Evidence for a Neuroticism-Linked Negative Schema. *Personality and Individual Differences*, *42*(7), 1221–1231.

Robinson, M. D., Ode, S., Wilkowski, B. M., & Amodio, D. M. (2007b). Neurotic contentment: a self-regulation view of neuroticism-linked distress. *Emotion*, *7*(3), 579–591.

Roelofs, K., van Peer, J., Berretty, E., Jong, P., Spinhoven, P., & Elzinga, B. M. (2009). Hypothalamus–Pituitary–Adrenal Axis Hyperresponsiveness Is Associated with Increased Social Avoidance Behavior in Social Phobia. *Biological Psychiatry*, *65*(4), 336–343.

Rolls, E. T. (1990). A Theory of Emotion, and its Application to Understanding the Neural Basis of Emotion. *Cognition & Emotion*, *4*(3), 161–190.

Rolls, E. T. (2004). The functions of the orbitofrontal cortex. *Brain and Cognition*, *55*(1), 11–29.

Rolls, E. T., & Grabenhorst, F. (2008). The orbitofrontal cortex and beyond: from affect to decision-making. *Progress in neurobiology*, *86*(3), 216–244.

Rombouts, S. A., Barkhof, F., Hoogenraad, F. G., Sprenger, M., & Scheltens, P. (1998). Within-subject reproducibility of visual activation patterns with functional magnetic resonance imaging using multislice echo planar imaging. *Magnetic Resonance Imaging*, *16*(2), 105–113.

Rousselet, G. A. (2012). Improving standards in brain-behavior correlation analyses. *Frontiers in Human Neuroscience.* 6, 1–11.

Roy, A. K., Shehzad, Z., Margulies, D. S., Kelly, A. M. C., Uddin, L. Q., Gotimer, K., Biswal, B. B., et al. (2009). Functional connectivity of the human amygdala using resting-state fMRI. *NeuroImage*, *45*(2), 614–626.

Roy, M., Shohamy, D., & Wager, T. D. (2012). Ventromedial prefrontal-subcortical systems and the generation of affective meaning. *Trends in Cognitive Sciences*, 16(3), 147–156

Rubino, V., Blasi, G., Latorre, V., Fazio, L., d'Errico, I., Mazzola, V., Caforio, G., et al. (2007). Activity in medial prefrontal cortex during cognitive evaluation of threatening stimuli as a function of personality style. *Brain Research Bulletin*, *74*(4), 250–257.

Rubinov, M., & Sporns, O. (2010). Complex network measures of brain connectivity: Uses and interpretations. *NeuroImage*, *52*(3), 1059–1069.

Russell, J. A. (1980). A circumplex model of affect. *Journal of Personality and Social Psychology*, *39*(6), 1161–1178.

Salamone, J. D. (1994). The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. *Behavioural Brain Research*, *61*(2), 117–133.

Salamone, J. D. (2009). Dopamine, Behavioral Economics, and Effort. *Frontiers in Behavioral Neuroscience*, *3*.

Sapolsky, R. M. (1994). *Why zebras don't get ulcers*. New York: WH Freeman.

Sareen, J., Campbell, D. W., Leslie, W. D., Malisza, K. L., Stein, M. B., Paulus, M. P., Kravetsky, L. B., et al. (2007). Striatal Function in Generalized Social Phobia: A Functional Magnetic Resonance Imaging Study. *Biological Psychiatry*, *61*(3), 396–404.

Schmidt, L. A., Santesso, D. L., Miskovic, V., Mathewson, K. J., McCabe, R. E., Antony, M. M., & Moscovitch, D. A. (2012). Test-retest reliability of regional electroencephalogram (EEG) and cardiovascular measures in social anxiety disorder (SAD). *International Journal of Psychophysiology*, 84(1), 65–73.

Schneider, T. R. (2004). The role of neuroticism on psychological and physiological stress responses. *Journal of Experimental Social Psychology*, *40*(6), 795–804.

Schommer, N., Kudielka, B., Hellhammer, D. H., & Kirschbaum, C. (1999). No evidence for a close relationship between personality traits and circadian cortisol rhythm or a single cortisol stress response. *Psychological Reports*, *84*(3), 840–842.

Schultz, W. (2004). Neural coding of basic reward terms of animal learning theory, game theory, microeconomics and behavioural ecology. *Current Opinion in Neurobiology*, *14*(2), 139–147.

Sedlmeier, P., & Gigerenzer, G. (1989). Do studies of statistical power have an effect on the power of studies? *Psychological Bulletin*, *105*(2), 309–316.

Sergerie, K., Chochol, C., & Armony, J. L. (2008). The role of the amygdala in emotional processing: A quantitative meta-analysis of functional neuroimaging studies. *Neuroscience & Biobehavioral Reviews*, *32*(4), 811–830.

Sheehan, D., Lecrubier, Y., Harnett Sheehan, K., Janavs, J., Weiller, E., Keskiner, A., Schinka, J., et al. (1997). The validity of the Mini International Neuropsychiatric Interview (MINI) according to the SCID-P and its reliability. *European Psychiatry*, *12*(5), 232–241.

Sierra-Mercado, D., Padilla-Coreano, N., & Quirk, G. J. (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology*, *36*(2), 529–538.

Simmons, J. P., Nelson, L. D., & Simonsohn, U. (2011). False-Positive Psychology: Undisclosed Flexibility in Data Collection and Analysis Allows Presenting Anything as Significant. *Psychological science*, *22*(11), 1359–1366.

Smith, S. M. (2012). The future of FMRI connectivity. *NeuroImage*, *62*(2), 1257–1266.

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004a). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*, S208–S219.

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004b). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*, S208–S219.

Smith, S. M., Miller, K. L., Salimi-Khorshidi, G., Webster, M., Beckmann, C. F., Nichols, T. E., Ramsey, J. D., et al. (2010). Network modelling methods for FMRI. *NeuroImage*, 54(2), 875–891.

Smits, D. J. M., & Boeck, P. D. (2006). From BIS/BAS to the big five. *European Journal of Personality*, *20*(4), 255–270.

Sotres-Bayon, F., & Quirk, G. J. (2010). Prefrontal control of fear: more than just extinction. *Current Opinion in Neurobiology*, *20*(2), 231–235.

Spinhoven, P., Elzinga, B. M., Hovens, J. G. F. M., Roelofs, K., Zitman, F. G., van Oppen, P., & Penninx, B. W. J. H. (2010). The specificity of childhood adversities and negative life events across the life span to anxiety and depressive disorders. *Journal of Affective Disorders*, *126*(1-2), 103–112.

Spinhoven, P., Elzinga, B., Roelofs, K., Hovens, J. G. F. M., van Oppen, P., Zitman, F. G., & Penninx, B. W. J. H. (2011). The effects of neuroticism, extraversion, and positive and negative life events on a one-year course of depressive symptoms in euthymic previously depressed patients versus healthy controls. *The Journal of nervous and mental disease*, *199*(9), 684–689.

Spreckelmeyer, K. N., Krach, S., Kohls, G., Rademacher, L., Irmak, A., Konrad, K., Kircher, T., et al. (2009). Anticipation of monetary and social reward differently activates mesolimbic brain structures in men and women. *Social Cognitive and Affective Neuroscience*, *4*(2), 158–165.

Stein, J. L., Wiedholz, L. M., Bassett, D. S., Weinberger, D. R., Zink, C. F., Mattay, V. S., & Meyer-Lindenberg, A. (2007a). A validated network of effective amygdala connectivity. *NeuroImage*, *36*(3), 736–745.

Stein, M. B., & Stein, D. J. (2008). Social anxiety disorder. *The Lancet*, *371*(9618), 1115–1125.

Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007b). Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *Am J Psychiatry*, *164*(2), 318–327.

Sutton, S. K., & Davidson, R. J. (1997). Prefrontal Brain Asymmetry: A Biological Substrate of the Behavioral Approach and Inhibition Systems. *Psychological science*, *8*(3), 204–210.

Tamir, M. (2005). Don't worry, be happy? Neuroticism, trait-consistent affect regulation, and performance. *Journal of Personality and Social Psychology*, *89*(3), 449–461.

Tamir, M. (2009). Differential Preferences for Happiness: Extraversion and Trait-Consistent Emotion Regulation. *Journal of Personality*, *77*(2), 447–470.

Tillfors, M., Furmark, T., Marteinsdottir, I., & Fredrikson, M. (2002). Cerebral blood flow during anticipation of public speaking in social phobia: a PET study. *Biological Psychiatry*, *52*(11), 1113–1119.

Tillfors, M., Furmark, T., Marteinsdottir, I., Fischer, H., Pissiota, A., Langstrom, B., & Fredrikson, M. (2001). Cerebral blood flow in subjects with social phobia during stressful speaking tasks: a PET study. *Am J Psychiatry*, *158*(8), 1220–1226.

Tom, S. M., Fox, C. R., Trepel, C., & Poldrack, R. A. (2007). The neural basis of loss aversion in decision-making under risk. *Science*, *315*(5811), 515–518.

Tottenham, N., Tanaka, J. W., Leon, A. C., McCarry, T., Nurse, M., Hare, T. A., Marcus, D. J., et al. (2009). The NimStim set of facial expressions: judgments from untrained research participants. *Psychiatry Research*, *168*(3), 242–249.

Trapnell, P. D., & Campbell, J. D. (1999). Private self-consciousness and the five-factor model of personality: Distinguishing rumination from reflection. *Journal of Personality and Social Psychology*, *76*(2), 284.

Trautmann, S. A., Fehr, T., & Herrmann, M. (2009). Emotions in motion: dynamic compared to static facial expressions of disgust and happiness reveal more widespread emotion-specific activations. *Brain Research*, *1284*, 100–115.

Turner, S. M., Beidel, D. C., Dancu, C. V., & Stanley, M. A. (1989). An empirically derived inventory to measure social fears and anxiety: The Social Phobia and Anxiety Inventory. *Psychological Assessment: A Journal of Consulting and Clinical Psychology*, *1*(1), 35–40.

Tye, K. M., Prakash, R., Kim, S.-Y., Fenno, L. E., Grosenick, L., Zarabi, H., Thompson, K. R., et al. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, *471*(7338), 358–362.

Tyrka, A. R., Mello, A. F., Mello, M. F., Gagne, G. G., Grover, K. E., Anderson, G. M., Price, L. H., et al. (2006). Temperament and hypothalamic-pituitary-adrenal axis function in healthy adults. *Psychoneuroendocrinology*, *31*(9), 1036–1045.

Tyrka, A. R., Wier, L. M., Anderson, G. M., Wilkinson, C. W., Price, L. H., & Carpenter, L. L. (2007). Temperament and response to the Trier Social Stress Test. *Acta Psychiatrica Scandinavica, 115*(5), 395–402.

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*, *10*(6), 397–409.

Urry, H. L., van Reekum, C. M., Johnstone, T., Kalin, N. H., Thurow, M. E., Schaefer, H. S., Jackson, C. A., et al. (2006). Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *Journal of Neuroscience*, *26*(16), 4415–4425.

Valentino, R. J., & Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European journal of pharmacology*, *583*(2-3), 194–203.

van der Wee, N. J., van Veen, J. F., Stevens, H., van Vliet, I. M., van Rijk, P. P., & Westenberg, H. G. (2008). Increased Serotonin and Dopamine Transporter Binding in Psychotropic Medication-Naive Patients with Generalized Social Anxiety Disorder Shown by 123I- -(4-Iodophenyl)-Tropane SPECT. *Journal of Nuclear Medicine*, *49*(5), 757–763.

van Duijvenvoorde, A., Zanolie, K., Zanolie, K., Rombouts, S. A. R. B., Raijmakers, M. E. J., & Crone, E. A. (2008). Evaluating the Negative or Valuing the Positive? Neural Mechanisms Supporting Feedback-Based Learning across Development. *The Journal of Neuroscience*, *28*(38), 9495–9503.

van Marle, H. J. F., Hermans, E. J., Qin, S., & Fernández, G. (2009). From Specificity to Sensitivity: How Acute Stress Affects Amygdala Processing of Biologically Salient Stimuli. *Biological Psychiatry*, *66*(7), 649–655.

van Marle, H. J. F., Hermans, E. J., Qin, S., & Fernández, G. (2010). Enhanced resting-state connectivity of amygdala in the immediate aftermath of acute psychological stress. *NeuroImage*, *53*(1), 348–354.

van Tol, M. J., van der Wee, N. J. A., van den Heuvel, O. A., Nielen, M. M. A., Demenescu, L. R., Aleman, A., Renken, R., et al. (2010). Regional Brain Volume in Depression and Anxiety Disorders. *Archives of general psychiatry*, *67*(10), 1002–1011.

Veer, I. M., Oei, N. Y. L., Spinhoven, P., van Buchem, M. A., Elzinga, B. M., & Rombouts, S. A. R. B. (2011). Beyond acute social stress: Increased functional connectivity between amygdala and cortical midline structures. *NeuroImage*, *57*(4), 1534–1541.

Veer, I. M., Oei, N. Y. L., Spinhoven, P., van Buchem, M. A., Elzinga, B. M., & Rombouts, S. A. R. B. (2012). Endogenous cortisol is associated with functional connectivity between the amygdala and medial prefrontal cortex. *Psychoneuroendocrinology*, *37*(7), 1039–1047.

Vertes, R. P. (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience*, *142*(1), 1–20.

Volman, I., Roelofs, K., Koch, S., Verhagen, L., & Toni, I. (2011). Anterior prefrontal cortex inhibition impairs control over social emotional actions. *Current Biology*, *21*(20), 1766–1770.

Vrtička, P., Andersson, F., Grandjean, D., Sander, D., & Vuilleumier, P. (2008). Individual Attachment Style Modulates Human Amygdala and Striatum Activation during Social Appraisal. *PLOS ONE*, *3*(8), e2868.

Vul, E., Harris, C., Winkielman, P., & Pashler, H. (2009). Puzzlingly high correlations in fMRI studies of emotion, personality, and social cognition. *Perspectives on Psychological Science*, *4*(3), 274–290.

Wager, T. D., & Nichols, T. E. (2003). Optimization of experimental design in fMRI: a general framework using a genetic algorithm. *NeuroImage*, *18*(2), 293–309.

Wager, T. D., Atlas, L. Y., Leotti, L. A., & Rilling, J. K. (2011). Predicting individual differences in placebo analgesia: contributions of brain activity during anticipation and pain experience. *Journal of Neuroscience*, *31*(2), 439–452.

Wager, T. D., Davidson, M. L., Hughes, B. L., Lindquist, M. A., & Ochsner, K. N. (2008). Prefrontal-Subcortical Pathways Mediating Successful Emotion Regulation. Neuron, *59*(6), 1037–1050.

Wager, T. D., Lindquist, M. A., Nichols, T. E., Kober, H., & Van Snellenberg, J. X. (2009a). Evaluating the consistency and specificity of neuroimaging data using meta-analysis. *NeuroImage, 45*(S1), S210–S221.

Wager, T. D., Lindquist, M., & Kaplan, L. (2007). Meta-analysis of functional neuroimaging data: current and future directions. *Social Cognitive and Affective Neuroscience*, *2*(2), 150–158.

Wager, T. D., Phan, K. L., Liberzon, I., & Taylor, S. F. (2003). Valence, gender, and lateralization of functional brain anatomy in emotion: a meta-analysis of findings from neuroimaging. *NeuroImage*, *19*(3), 513–531.

Wager, T. D., van Ast, V. A., Hughes, B. L., Davidson, M. L., Lindquist, M. A., & Ochsner, K. N. (2009b). Brain mediators of cardiovascular responses to social threat, Part II: Prefrontal-subcortical pathways and relationship with anxiety. *NeuroImage*, 47(3), 836-851.

Wager, T. D., Waugh, C. E., Lindquist, M., Noll, D. C., Fredrickson, B. L., & Taylor, S. F. (2009c). Brain mediators of cardiovascular responses to social threat: part I: Reciprocal dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity. *NeuroImage*, *47*(3), 821–835.

Warwick, J. M., Carey, P. D., Cassimjee, N., Lochner, C., Hemmings, S., Moolman-Smook, H., Beetge, E., et al. (2012). Dopamine transporter binding in social anxiety disorder: the effect of treatment with escitalopram. *Metabolic brain disease*, *27*(2), 151–158.

Watson, D., Clark, L. A., & Harkness, A. R. (1994). Structures of personality and their relevance to psychopathology. *Journal of Abnormal Psychology, 103*(1), 18–31.

Waugh, C. E., Hamilton, J. P., Chen, M. C., Joormann, J., & Gotlib, I. H. (2012). Neural temporal dynamics of stress in comorbid major depressive disorder and social anxiety disorder. *Biology of mood & anxiety disorders*, *2*(1), 1-15.

Waugh, C. E., Wager, T. D., Fredrickson, B. L., Noll, D. C., & Taylor, S. F. (2008). The neural correlates of trait resilience when anticipating and recovering from threat. *Social Cognitive and Affective Neuroscience, 3*(4), 322–332.

Weeks, J. W., Heimberg, R. G., Fresco, D. M., Hart, T. A., Turk, C. L., Schneier, F. R., & Liebowitz, M. R. (2005). Empirical Validation and Psychometric Evaluation of the Brief Fear of Negative Evaluation Scale in Patients With Social Anxiety Disorder. *Psychological Assessment*, *17*(2), 179–190.

Whalen, P. J. (2007). The uncertainty of it all. *Trends in Cognitive Sciences*, *11*(12), 499–500.

Williams, L. M. (2006). Mode of Functional Connectivity in Amygdala Pathways Dissociates Level of Awareness for Signals of Fear. *Journal of Neuroscience*, *26*(36), 9264–9271.

Winter, K. A., & Kuiper, N. A. (1997). Individual differences in the experience of emotions. *Clinical Psychology Review, 17*(7), 791–821.

Wright, C. I., Feczko, E., Dickerson, B., & Williams, D. (2007). Neuroanatomical correlates of personality in the elderly. *NeuroImage*, *35*(1), 263–272.

Wright, C. I., Williams, D., Feczko, E., Barrett, L. F., Dickerson, B. C., Schwartz, C. E., & Wedig, M. M. (2006). Neuroanatomical Correlates of Extraversion and Neuroticism. *Cerebral Cortex*, *16*(12), 1809–1819.

Yarkoni, T. (2009). Big Correlations in Little Studies: Inflated fMRI Correlations Reflect Low Statistical Power-Commentary on Vul et al. (2009). *Perspectives on Psychological Science*, *4*(3), 294–298.

Yarkoni, T., Poldrack, R. A., Nichols, T. E., Van Essen, D. C., & Wager, T. D. (2011). Large-scale automated synthesis of human functional neuroimaging data. *Nature methods*, *8*(8), 665–670.

Yarkoni, T., Poldrack, R. A., Van Essen, D. C., & Wager, T. D. (2010). Cognitive neuroscience 2.0: building a cumulative science of human brain function. *Trends in Cognitive Sciences*, *14*(11), 489–496.

Zelenski, J. M., & Larsen, R. J. (1999). Susceptibility to affect: a comparison of three personality taxonomies. *Journal of Personality*, *67*(5), 761–791.

Zuo, X.-N., Di Martino, A., Kelly, C., Shehzad, Z. E., Gee, D. G., Klein, D. F., Castellanos, F. X., et al. (2010a). The oscillating brain: complex and reliable. *NeuroImage*, *49*(2), 1432–1445.

Zuo, X.-N., Kelly, C., Adelstein, J. S., Klein, D. F., Castellanos, F. X., & Milham, M. P. (2010b). Reliable intrinsic connectivity networks: Test–retest evaluation using ICA and dual regression approach. *NeuroImage*, *49*(3), 2163–2177.

List of Abbreviations

ACC	Anterior Cingulate Cortex
ANS	Autonomic Nervous System
BLA	Basolateral Amygdala
BAS	Behavioral Activation System
BIS	Behavioral Inhibition System
BOLD	Blood Oxygen Level Dependent
CeA	Central Amygdala
CER	Cognitive Emotion Regulation
CRH	Corticothrophin Releasing Hormone
DSM-IV	Diagnostic & Statistical Manual for mental disorders IV
DA	Dopamine
ECG	Electrocardiography
fMRI	functional Magnetic Resonance Imaging
HPA	Hypothalamic-Pituitary-Axis
LC	Locus Coeruleus
MPFC	medial Prefrontal Cortex
NESDA	Netherlands Study of Depression and Anxiety
OFC	Orbital Frontal Cortex
PAG	Periaquaductal Grey
PPI	Psycho Physiological Interaction
ROI	Region of Interest
RS	Resting-State
SAD	Social Anxiety Disorder
VS	Ventral Striatum
VTA	Ventral Tegmental Area
VMPFC	ventromedial Prefrontal Cortex
VBM	Voxel Based Morphometry

Nederlandse Samenvatting

Mensen verschillen in de mate waarin zij sociale situaties waarnemen en verdragen. Sociale-angststoornis (SA) is de extreme angst voor de beoordeling door anderen. De stoornis wordt sinds 40 jaar onderscheiden van andere specifieke angsten of agorafobie. Dit proefschrift onderzoekt de neurobiologie van SA en persoonlijkheidsfactoren die geassocieerd zijn met de ontwikkeling ervan.

Hoofdstuk 1 behandelt de algemene achtergrond van het onderzoek in dit proefschrift. SA wordt gekenmerkt door een aanhoudende angst voor situaties waarin men kan worden beoordeeld door anderen. Iemand met SA is bang dat hij of zij zal handelen op een manier die vernederend of beschamend zal zijn. De blootstelling aan de gevreesde sociale situaties gaat bijna altijd gepaard met extreme angst en mogelijk met paniekaanvallen. Sociale situaties worden daarom vaak vermeden of slechts met grote angst of stress doorstaan. Als deze angst in belangrijke mate de normale dagelijkse routine verstoord wordt de diagnose van SA gesteld. SA komt bij ongeveer vijf tot twaalf procent van de mensen voor. Alhoewel er behoorlijk succesvolle behandelingen bestaan, in de vorm van gedragstherapie en van farmacologische interventies, reageert ongeveer 40 procent van de mensen niet op deze therapieën. Fundamenteel onderzoek naar de neurobiologie van SA heeft als doel de onderliggende mechanismen van SA te begrijpen. Dit kan tevens leiden tot verbetering van behandelingen van SA.

Persoonlijkheidstheorieën beschrijven vaak persoonlijkheidskenmerken die geassocieerd worden met de beleving van bepaalde emoties. Vaak wordt onderscheid gemaakt tussen persoonlijkheidskenmerken die gerelateerd zijn aan een frequenter/intensievere beleving van positieve of negatieve emoties. Extraversie en neuroticisme zijn de bekendste voorbeelden van dergelijke persoonlijkheidskenmerken. Deze persoonlijkheidskenmerken zijn ook zeer relevant voor de ontwikkeling van sociale angst. Extraversie wordt gezien als een beschermende factor in de ontwikkeling van SA: hoe extraverter mensen zijn, hoe kleiner de kans op de ontwikkeling van SA. Het omgekeerde geldt voor neuroticisme: hoe hoger iemand scoort op neuroticisme - wat vaak gepaard gaat met verhoogde angst en piekeren - hoe groter de kans op de ontwikkeling van sociale angst. Naast deze factoren zijn de sociale omgeving waarin iemand is opgegroeid (waaronder de rol van de ouders) en het meemaken van traumatische gebeurtenissen zeer belangrijk voor de vatbaarheid voor het ontwikkelen van SA.

Er zijn veel verschillende hersengebieden betrokken bij sociale angst en de persoonlijkheidskenmerken neuroticisme en extraversie. Wij richten ons hier op centrale neurale circuits: gekoppelde

hersenstructuren die samen een bepaalde cognitieve of emotionele functie vervullen. Een breed onderscheid wordt hier gemaakt tussen het *emotiecircuit* (dit circuit centreert zich rond de amygdala, de hersenstamnuclei en prefrontale schors) en het *motivatiecircuit* (gecentreerd rond het striatum, het ventraal tegmentale gebied, en ook de prefrontale schors). Het emotiecircuit is essentieel voor de detectie van belangrijke, nieuw stimuli in de omgeving, om daar vervolgens snel op te kunnen reageren. Het motivatiecircuit is cruciaal voor het detecteren van mogelijk belonende situaties, en om gedrag voor te bereiden om de beloning te behalen. Aan de andere kant is dit circuit ook belangrijk bij het vermijden van straf. De emotie- en motivatiecircuits vormen de basis voor vele belangrijke cognitief-emotionele functies, en de verstoring van hun functie is gekoppeld aan de ontwikkeling van onder andere angststoornissen. Bij het in kaart brengen van hersenfuncties wordt in dit onderzoek gebruik gemaakt van de beeldvormende techniek *functional Magnetic Resonance Imaging* (fMRI). Hiermee kan op (een indirecte manier) het functioneren van de hersenen worden gemeten wanneer deelnemers in een MRI-scanner een taak uitvoeren. Het functioneren van de hersenen kan op verschillende manieren worden benaderd. Het kan gaan om de lokale activatie van een bepaald hersengebied, het volume van een hersenstructuur, en de communicatie tussen verschillende hersenstructuren. Het centrale idee bij veel psychiatrische aandoeningen is dat met name de communicatie tussen zogenaamde regulerende prefrontale/corticale gebieden en subcorticale gebieden (zoals de amygdala) is verstoord.

Het doel van het huidige onderzoek is om deze neurale circuits te bestuderen in relatie tot sociale angst en persoonlijkheidskenmerken die belangrijk zijn voor de ontwikkeling van sociale angst. De deelnemers aan dit onderzoek hebben taken uitgevoerd die het functioneren van de twee neurale circuits beogen te testen. De verwachting is dat patiënten met sociale angst een ander activatiepatroon vertonen in het emotiecircuit dan de controlegroep. Dit zal een combinatie zijn van een sterker reactieve amygdala en verminderde prefrontale controle op sociale stimuli. Wat betreft het motivatiecircuit verwachten wij dat de activatiebalans bij SA is verstoord, en SA deelnemers sterker reageren op het vermijden van een negatieve beoordeling dan op het behalen van een positieve beoordeling. Wat betreft neuroticisme en extraversie is de verwachting dat deze persoonlijkheidsfactoren samenhangen (correleren) met het functioneren van de twee neurale circuits. Neuroticisme zal voornamelijk gepaard gaan met verhoogde reactiviteit van het emotie circuit en verminderde prefrontale regulatie, en sterkere vermijdingsdrang. Extraversie is gekoppeld aan sterkere prefrontale controle en beloningsgevoeligheid. De verwachting is daarom dat binnen de controlegroep bij deelnemers die hoog op neuroticisme en/of laag op extraversie scoren, deze circuits functioneren zoals bij de deelnemers met sociale angst.

De data uit Hoofdstuk 2 en 3 zijn onderdeel van een groot longitudinaal onderzoeksproject: de Nederlandse studie naar depressie en angst (NESDA). in hoofdstuk 2 wordt de link met neuroticisme en amygdala-prefrontale connectiviteit onderzocht. Dit zijn twee belangrijke structuren in het emotiecircuit. De amygdala is belangrijk voor het verwerken van emotionele stimuli in de omgeving. De prefrontale schors is belangrijk voor het reguleren, en interpreteren van deze reacties. De deelnemers aan het onderzoek kregen emotionele gezichtsuitdrukkingen te zien, terwijl zij in de MRI scanner lagen. Op deze manier kon worden onderzocht hoe mensen reageren op negatieve (bijvoorbeeld boze) gezichtsuitdrukkingen, in vergelijking met hun reactie op neutrale gezichtsuitdrukkingen. De belangrijkste bevinding was dat de connectiviteit tussen de amygdala en een regulerend deel van de prefrontale schors (de rostrale anteriore cingulate cortex) verminderde naarmate de deelnemers hoger scoorden op neuroticisme. Daarnaast was de connectiviteit tussen de amygdala en het "evaluerende deel" van de prefrontale schors (de dorsomediale schors) juist positief gecorreleerd met neuroticisme. Dat zou kunnen betekenen dat mensen die hoger scoren op neuroticisme, zowel meer moeite hebben negatieve gezichtsuitdrukkingen in hun omgeving te verwerken of te controleren, als ook deze sneller te interpreteren als een negatieve beoordeling op het eigen handelen. De combinatie van deze twee resultaten geeft inzicht in de kwetsbaarheid voor negatieve stimuli geassocieerd met neuroticisme.

Hoofdstuk 3 richt zich op het volume van hersengebieden in het emotie- en motivatiecircuit. Het volume van hersengebieden kan informatie geven over de onderliggende processen die belangrijk zijn voor hersenactivatie. Naast neuroticisme is in dit onderzoek vooral ook de rol van extraversie benadrukt. De belangrijkste bevindingen waren dat zowel de amygdala als de orbitofrontale cortex een positieve correlatie met extraversie lieten zien. In tegenstelling tot de verwachtingen was er geen significante correlatie tussen hersenvolume en neuroticisme. De amygdala en de orbitofrontale cortex zijn beide belangrijk voor het verwerken van emotionele informatie. Dit kan er op wijzen dat hoe extraverter iemand is, hoe meer (regulerend) vermogen hij heeft om emotionele stimuli te verwerken.

Hoofdstuk 4 beschrijft een onderzoek naar het motivatiecircuit van patiënten met sociale angst. De hypothese was dat mensen met sociale angst een sterkere motivatie hebben om straf te vermijden dan om een beloning te behalen. Dit effect zou tot uitdrukking moeten komen in onbalans van de activatie in het neurale motivatie systeem. Zowel deelnemers met sociale angst als deelnemers van

de controlegroep voerden een taak uit waarin zij ofwel "beloond" werden met de vertoning van een blij gezicht als zij een detectietaak zo snel mogelijk hadden uitgevoerd, ofwel "gestraft" werden met de vertoning van een boos gezicht als de detectietaak niet snel genoeg werd uitgevoerd. De resultaten lieten zien dat de SA-groep inderdaad een relatief sterker actief striatum (een belangrijk onderdeel van het motivatiecircuit) hebben als straf kan worden ontlopen. Dit wijst op het sterke vermijdingsgedrag dat zo kenmerkend is voor SA.

In Hoofdstuk 5 wordt ook een onderzoek beschreven waar de anticipatie van sociale situaties in sociale angst is gemeten, maar nu in een meer direct stressvolle context. In dit onderzoek voerden de deelnemers geen taakje uit, maar lagen zij voor een aantal minuten stil in de MRI scanner (resting-state fMRI). Na een zogenaamde baseline-meting kregen de deelnemers te horen dat zij na het fMRI onderzoek, geacht werden een korte presentatie te geven, waar de onderzoekers als beoordelaars zouden optreden. Na deze mededeling volgde een nieuwe fMRI-meting, waarin dus geen specifieke taak werd uitgevoerd maar waarin deelnemers wel verhoogde angst en spanning rapporteerden. Deze meting werd gevolgd door de mededeling dat de korte presentatie toch niet doorging en het onderzoek na de laatste fMRI-scan, klaar zou zijn. Op deze manier werden twee "rustmetingen" en een stressmeting gedaan. Tijdens de stressmeting vertoonde de SA-groep een verhoogde hartslag en stressbeleving. Dat ging gepaard met minder sterke connectiviteit tussen corticale emotie regulerende gebieden en de amygdala. Deze bevindingen kunnen een neurobiologische verklaring vormen voor de grote angst die SA patiënten ervaren als zij in het openbaar moeten spreken.

In Hoofdstuk 6 zijn de data van het onderzoek van hoofdstuk 5 opnieuw geanalyseerd maar nu met nadruk op de persoonlijkheidsfactoren neuroticisme en extraversie. Evenals in hoofdstuk 2 en 3 hebben wij ons hier nadrukkelijk op de controledeelnemers gericht en individuele verschillen binnen deze groep onderzocht. Uit de resultaten bleek dat hoe hoger mensen op neuroticisme scoren, hoe zwakker de connectiviteit tussen de corticale emotie-regulatiegebieden en de amygdala werd. Deze relatie was echter niet afhankelijk van het al dan niet in afwachting zijn van spreken in het openbaar. Daarentegen vertoonde extraversie juist wel een effect met corticale-amygdala-connectiviteit die afhankelijk was van de stressanticipatie. Gedurende de stressperiode lieten sterker extraverte mensen meer prefrontale controle over de amygdala zien. De combinatie van de extraversie- en neuroticisme-bevindingen verschaffen inzicht in de neurobiologie van persoonlijkheidsfactoren die mensen meer of minder vatbaar kunnen maken voor de ontwikkeling van onder andere sociale angst.

Hoofdstuk 7 behandelt statistische problemen die een grote rol spelen bij veel fMRI-onderzoek naar het functioneren van de hersenen van mensen met psychische aandoeningen, en meer in het algemeen bij fMRI-onderzoek naar verschillen tussen individuen. Het meest problematisch zijn de combinatie van lage statistische power en de grote flexibiliteit in data-analyse opties. Daarnaast wordt in wetenschappelijke publicaties van fMRI-gegevens weinig nadruk gelegd op onafhankelijke kwantitatieve replicatie van bevindingen. De combinatie van deze factoren leidt ertoe dat het bijna onmogelijk is om de waarde van gepubliceerde resultaten te beoordelen. Dit heeft te maken met ten minste twee consequenties van de statistische problemen. Ten eerste kan door de grote flexibiliteit in data-analyse-onderzoek de misleidende suggestie worden gewekt dat er bevindingen zijn gedaan die in overeenstemming zijn met eerder onderzoek. Aan de andere kant kan door het gebrek aan statistische power en de gerelateerde grote invloed van steekproeffouten een onnodig verwarrend beeld ontstaan als verschillende onderzoeken naar hetzelfde onderwerp activatie in andere hersengebieden laten zien. Theoretisch kunnen deze verschillen in hun geheel toe te schrijven zijn aan de toevallige samenstelling van de steekproef. Het is waarschijnlijk dat de huidige gepubliceerde literatuur een moeilijk te doorgronden combinatie is van deze twee uitwerkingen. Het is daarom aan te bevelen in dat toekomstig onderzoek zal bestaan uit grote steekproeven (hogere statistische power) en idealiter wordt gecombineerd met onafhankelijk replicatie onderzoek.

Hoofdstuk 8 geeft een samenvatting van de belangrijkste bevindingen uit hoofdstuk 2 tot en met 7. De hoofdstukken 2, 3 en 6 tonen consistent aan dat individuele verschillen in de persoonlijkheidskenmerken neuroticisme en extraversie gepaard gaan met verschillen in het functioneren van de emotie- en motivatiecircuits. Met met name de communicatie tussen prefrontale en subcorticale gebieden zoals de amygdala lijkt hier van belang. Neuroticisme correleert met verminderde, en extraversie met verhoogde prefrontale regulatie van de amygdala. Dit zijn belangrijke bevindingen omdat deze persoonlijkheidskenmerken ook geassocieerd zijn met de ontwikkeling van onder andere sociale angst en dit kan duiden op een neurobiologische kwetsbaarheid voor de ontwikkeling van sociale angst.

De hoofdstukken 4 en 5 demonstreren dat zich bij patiënten met sociale angst veranderingen manifesteren in het emotie- en motivatiecircuit. SA-patiënten zijn, vergeleken met de controlegroep, meer gemotiveerd om een straf te ontlopen als daartoe de kans bestaat (hoofdstuk 4). In een situatie die een sterkere mate van stress opwekt, (hoofdstuk 5) zien we een verminderde regulatie van de amygdala door corticale gebieden. Een cruciale vraag die zich opdringt, is of vermijdingsgeneigdheid

in de experimentele taak samenhangt met verhoogde stressgevoeligheid. Nadere analyse toonde dat deze effecten inderdaad in zekere mate samenhangen: hoe sterker de striatale reactiviteit om een straf te vermijden (Hoofdstuk 4), hoe sterker de stressreactie (hartslagverhoging, en stressbeleving) tijdens het in afwachting zijn in de aanloop naar het spreken in het openbaar (hoofdstuk 5).

Het is belangrijk om te vermelden dat het onderzoek in dit proefschrift, evenals het meeste onderzoek naar klinische populaties in de literatuur, eenmalige metingen betreft. Dit is een belangrijke tekortkoming, omdat de conclusies die kunnen worden getrokken, hierdoor zeer worden beperkt. Met name wat betreft de relatie tussen persoonlijkheidskenmerken en de ontwikkeling van sociale angst, en mogelijke andere stoornissen, is zogenaamd longitudinaal onderzoek essentieel. Zo'n onderzoek vereist waarschijnlijk een grote steekproef. Het NESDA-project beschikt over een grote hoeveelheid gegevens en herhaalde metingen, en daar liggen mogelijkheden om vragen rond de ontwikkeling (verandering) van aandoeningen en/of symptomen te onderzoeken.

Het onderhavige onderzoek probeert inzicht te verschaffen in de neurale mechanismen betrokken bij sociale angst en relevante persoonlijkheidskenmerken. De bevindingen duiden op structurele en functionele verschillen in het emotie- en motivatiecircuit. Patiënten met SA vertoonden een combinatie van verhoogde vermijdingsgevoeligheid en verminderde corticale-subcorticale regulatie tijdens stress. Neuroticisme leek voornamelijk gekoppeld aan verminderde neurale regulatie tijdens niet-stressvolle situaties terwijl extraversie juist meer specifiek betrokken was bij verhoogde regulatie tijdens stress. Het functioneren van deze circuits lijkt dus zeer belangrijk voor SA en de ontwikkeling daarvan. Voor de resultaten van dit onderzoek geldt - evenals voor al het bestaande gepubliceerde onderzoek - dat onafhankelijke replicaties nodig zijn om te bepalen of effecten vals dan wel echt positief zijn.

Dankwoord / Acknowledgement

Na een paar jaar aan een onderzoek werken, zijn er een hele hoop mensen om te bedanken.

Ten eerste Karin. Ik stond op het punt naar Stockholm te vertrekken, maar daar heb jij me van weerhouden. Ik heb daar nooit spijt van gehad. Ik had me geen andere begeleider kunnen voorstellen die zo gedreven is, begaan met onderzoek, en alle nieuwe onderzoek- en analyseplannen altijd met zoveel enthousiasme steunt. Bedankt voor alles!

Daarnaast dank ook Philip en Serge voor de begeleiding, zeker in het begin van het onderzoek, en het getoonde vertrouwen. André, bedankt voor de samenwerking op het nesda project.

Veel dank natuurlijk aan alle deelnemers aan het onderzoek, en de behandelcentra die hebben meegewerkt: Bert van Klaveren van Duin en Bollen GGZ, en speciale dank aan Ed Berretty, Barbara Erenstein en alle andere collega's van PsyQ voor de inzet bij de werving en een zeer geslaagde samenwerking.

Dank aan collega's op de verschillende afdelingen waar ik heb gewerkt. In Groningen: Ruud, Remco, Johan, Katharina. In Leiden: Anne-Laura, Marc, Nic, Rimke, Sander, Anne-Wil, Esther, Maarten, Yvette, Hilal, Floor, Niki, Josanne, Bart, Bernet, Willem, Marieke, Mumtaz, Jolijn, Jos, Elsbeth. In Nijmegen: Eni, Mike, Marcella, Gero, Maartje, Linda, Joyce, Wies, Maria. Dank natuurlijk aan iedereen van het EPAN lab, bedankt voor alle discussies over het onderzoek en gezellige uitjes, Peter, Jacobien, Inge, Verena, Bernd, Mariska, Henk van Steenbergen. Dank ook aan alle studenten die hebben meegewerkt aan het sociale angst onderzoek: Jorien, Verena, Marjolijn, Miranda, Mi Mi.

Ramona, thank you for our collaboration on the NESDA data. Dorien, we zijn samen begonnen aan onze AIO onderzoeken, het rekruteren van de patiënten was een enorme klus, maar het is ons toch maar mooi gelukt! Bedankt voor de samenwerking. Marie-Jose, bedankt voor onze samenwerking op het onderzoek naar persoonlijkheid en hersenstructuur en voor de korte, maar heel gezellige tijd als kamergenoten. Muriel, bedankt voor de geslaagde samenwerking op het "imagery" experiment. Nicole, bedankt voor de gezelligheid, discussies over corrected p values en mediatie analyses (vooral dat laatste).

Ilya, het is heel simpel, zonder jouw kennis van de LIBC scanner, FSL analyses, en talent in experimenteel onderzoek had het sociale angst project niet kunnen plaatsvinden. Daarnaast konden we elkaar ook nog eens behoorlijk verdragen in de kroeg, wat de afgelopen jaren tot een zeer geslaagde samenwerking heeft gemaakt.

Keegan, de best Nederlands sprekende Amerikaan allertijden en buurman bij FSW. Altijd gezellig om even langs te lopen. Ik snapte nooit zo veel van die gezondheidsinterventies en mediatie modellen, en jij niet van hersenonderzoek. We begrepen elkaar beter over basketbal (beste dunk allertijden: Jason Richardson 2003) en andere belangrijke zaken. We worden ook nog steeds gemist op de tribune bij zz Leiden.

I am very grateful to Tor Wager for our collaboration, the warm welcome I received in your lab, and your advice on analyses and interpretation of results. Many thanks also to the other lab members, Yoni, Scott, Jess, Luca, Marieke, Wani, So Young. Special thanks to Tal for the critical thinking and help with the data analysis, but most importantly for explaining me what statistical power really means, my views on fMRI research have not been the same since. Melanie, thanks for all the great times and support while I was working on the analyses.

I would like to thank my new colleagues at the University of Chicago: Sarah, Emil, Kristen, Royce, Rose, and Margaret, for the kind reception and support while I was starting a new job and finishing my thesis.

Dank aan mijn goede vrienden voor de vele jaren gezelligheid. Voor sommige al sinds de basisschool: Jasper, Sicco, Wouter (gezellig dat jullie naar Chicago komen). In Utrecht: Bram, Breg, Leontien, Hugo, Bert, Peter, Kim, Saskia, Nils en D'Shawn. Don, hockeykoning uit Limburg, gaaf dat jij ook naar Chicago komt binnenkort. In New York: Saskia. Speciale dank aan Roel voor het omtoveren van al mijn matige posters, figuren, website en nu ook het proefschrift, tot kleine kunstwerkjes. Maar veel belangrijker nog, bedankt voor de vele, vele goede tijden.

Dank aan Agnes, mijn lieve kleine zusje (dat zal altijd zo blijven), Haico, en mijn toekomstige nichtje.

Grote dank aan mijn ouders, Eva en Crit, voor de onvoorwaardelijke steun, zorg en liefde zolang ik mij kan herinneren en daarvoor. Er is alleen een nadeel: met zulke ouders geldt voor mijzelf helaas wel het statistische principe van regressie naar het gemiddelde.

Biography

Henk Cremers was born on September 5th, 1981 in Groningen, The Netherlands. He obtained a BSC in Psychology from Utrecht University in 2005 and an MSC in Cognitive Neuroscience from the Radboud University Nijmegen in 2007. The next year, Henk started his PHD project on Social Anxiety at the Clinical Psychology Department of Leiden University, supervised by Karin Roelofs, Serge Rombouts and Philip Spinhoven. The project moved to the Radboud University Nijmegen in 2011. Henk visited the lab of Tor Wager at the University of Colorado in 2011 for a collaboration on the FMRI project on stress in social anxiety. Since November 2012, Henk works as a postdoctoral scholar at the Department of Psychiatry of the University of Chicago, with Sarah Keedy and Emil Coccaro.

Publications

Cremers, H.R., van Tol, M.-J., Roelofs, K., Aleman, A., Zitman, F. G., van Buchem, M. A., Veltman, D. J., van der Wee, N. J. (2011). Extraversion Is Linked to Volume of the Orbitofrontal Cortex and Amygdala. *PLOS ONE*, *6*(12).

van Wingen, G. A., van Eijndhoven, P., **Cremers, H. R.,** Tendolkar, I., Verkes, R. J., Buitelaar, J. K., & Fernández, G. (2010). Neural state and trait bases of mood-incongruent memory formation and retrieval in first-episode major depression. *Journal of Psychiatric Research*, *44*(8), 527–534.

Cremers, H. R., Demenescu, L. R., Aleman, A., Renken, R., van Tol, M.-J., van der Wee, N. J. A., Veltman, D. J., Roelofs, K. (2010). Neuroticism modulates amygdala—prefrontal connectivity in response to negative emotional facial expressions. *NeuroImage*, 49(1), 963-970.

In preparation

Cremers, H.R., Veer, I.M., Spinhoven, P., Rombouts, S.A.R.B., Yarkoni, T., Wager, T.D., Roelofs, K. (submitted). Dysfunctional cortical-amygdala coupling in social anxiety disorder during the anticipation of giving a public speech.

Cremers, H.R., Veer, I.M., Spinhoven P., Rombouts, S.A.R.B., Roelofs, K. (Submitted). Neural sensitivity to social punishment versus reward anticipation in social anxiety disorder.