The embarrassed brain: towards a neurobiology of generalized social anxiety disorder

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Summary and discussion
Summary

Social anxiety disorder (SAD) is characterized by a persistent fear of one or more social or performance situations in which the person is exposed to people or to possible scrutiny by others. Two subtypes of SAD can be distinguished: the specific (sSAD) and the generalized type (gSAD). In this thesis, we chose to investigate gSAD, since it is the most disabling, most severe and complete form, showing all aspects of social anxiety. gSAD is associated with impairment of functioning in social life, work and family life. An epidemiological survey in Ontario, Canada, which discriminated between the specific (sSAD) and the generalized type (gSAD), reported life time prevalence rates for gSAD of 5.9%. Treatments of choice for gSAD are serotonin reuptake inhibitors (SSRIs), serotonin-noradrenalin reuptake inhibitors (SNRIs) and cognitive behavioural therapy (CBT). The underpinnings of the neurobiology of gSAD are not clear yet, but important to investigate for the development of new treatments. Research in other affective disorders indicates that several hormonal and neuroendocrine systems might be involved, such as the serotonergic system, the dopaminergic system, and both branches of the stress system (the autonomic nervous system (ANS)) and the hypothalamic-pituitary-adrenal-axis (HPA-axis). Female gonadal hormones also influence affective symptoms and thus may be involved in affective disorders. The aim of this thesis is to test the role of these hormones and neurotransmitter systems that influence the brain for changes and investigate their involvement in the neurobiology of gSAD.

Generalized social anxiety disorder (gSAD) and Panic Disorder (PD) are among the most prevalent anxiety disorders. Although the two disorders have a different core phenomenology, with spontaneous panic attacks in PD, and fear of scrutiny by others in gSAD, data from epidemiological, pharmacotherapeutical, genetic and neurobiological studies suggest a possible overlap in the neurobiology of gSAD and PD. In chapter 2 we directly compared the behavioural, neuroendocrine and physiological effects to an acute serotonergic challenge in seven (five male and two female) patients with gSAD, PD and healthy controls, pair wise matched on age and sex. For this challenge we used the rapid intravenous administration of 0.1 mg/kg meta-chlorophenylpiperazine (m-CPP), a (partial) 5-HT$_{2c}$ receptor agonist that also possesses moderate to low affinity for other 5-HT receptors, as well as for ($\alpha_1$) adrenergic and dopamine receptors. In the study no lifetime comorbidity was allowed. Behavioural responses to m-CPP were measured with a Visual Analogue Scale for anxiety and the Panic Symptom Scale. After the challenge the occurrence of panic attacks according to DSM-IV criteria was assessed. Furthermore, temperature, blood pressure, heart rate, cortisol and growth hormone responses to the challenge were assessed. Panic attacks were significantly more frequently provoked in patients with PD (85%), than in patients with gSAD (14%) and to healthy controls (0%). Effects on the other behavioural parameters, but not on the neuroendocrine and physiological parameters, were significantly greater in patients with PD compared to patients with gSAD and controls. The difference in responses on m-CPP in PD and gSAD suggest that PD and gSAD are distinct psychiatric disorders on a neurobiological level. These results might also support involvement of the serotonergic system in gSAD. We explored this further in a study using SPECT.
In chapter 3, we examined the $^{123}$I-ß-(4-iodophenyl)-tropane ($^{123}$I-ß-CIT) binding potential for the serotonin and dopamine transporters using SPECT imaging in patients with gSAD and in age and gender matched healthy controls. Twelve psychotropic medication–naïve patients with social anxiety disorder, generalized subtype (5 women and 7 men) and 12 healthy controls were studied. The SPECT scans for the serotonin transporter binding were made four hours and for the dopamine transporter binding 24 hours after the infusion of $^{123}$I-ß-CIT.Volumes of interest were constructed on MRI-coregistered SPECT scans. We found significantly higher binding potentials for the serotonin transporter in the left and right thalamus of gSAD patients. Patients had also a significantly higher binding potential for the dopamine transporter in the striatum. The present study provides direct evidence for abnormalities in not only the serotonergic, but also the dopaminergic system in patients with gSAD.

The efficacy of mirtazapine on gSAD symptoms was studied in chapter 4. Mirtazapine is an antidepressant that blocks α₂-adrenergic autoreceptors, resulting in the stimulation of both noradrenergic and serotonergic pathways. It also blocks 5-HT$_2$ and 5-HT$_3$ receptors, and has antihistaminergic properties. Studies in other anxiety disorders suggest that mirtazapine has anxiolytic properties. We studied the effects of mirtazapine 30 mg during 12 weeks in fourteen gSAD patients without axis I comorbidity according to DSM-IV criteria. Twelve patients completed the study. Two patients (14.3%) dropped out due to side-effects. Generally, mirtazapine was well tolerated. Five out of 12 patients (41.7%) were classified as responders, based on a Clinical Global Improvement score of 1 or 2 and a reduction of the Liebowitz Social Anxiety Scale (LSAS) of 40%. The mean total score on the LSAS, as well as the anxiety and avoidance subscores, decreased significantly. This open pilot study suggests that further investigations are warranted to prove the efficacy of mirtazapine in generalized social anxiety disorder. The study also suggests an involvement of the serotonergic, noradrenergic and histaminergic pathways in gSAD. 

In chapter 5 we describe both HPA-axis and ANS activity in basal non-challenging conditions and after 0.5 mg dexamethasone in gSAD patients, to test the reactivity of the stress-system. To ensure stress-free sampling we collected saliva (not blood as it requires a stressful venipuncture) and determined cortisol and alpha-amylase (sAA), the latter a relative new marker of autonomic activity. Forty-three untreated gSAD patients without comorbidity were compared with 43 age and gender matched healthy controls in non-stressed conditions on sAA and cortisol after awakening, during the day (including late evening), and after a low dose (0.5 mg) of dexamethasone. Apart from the assessments in the morning, gSAD patients had significantly higher diurnal and post-dexamethasone 1600 h sAA levels. No differences between gSAD and controls in any cortisol measurements were found. In conclusion, in gSAD in basal, non-stimulated conditions and after dexamethasone, we found hyperactivity of the ANS, as measured with sAA, but not of the HPA-axis. This suggests a relative increased activity of the ANS but not of the HPA-axis. The hyperactivity of the ANS is in line with the clinically observed somatic symptoms of hyperarousal in gSAD such as trembling, blushing and perspiration.
In chapter 6 we studied the interplay between the serotonergic system and the stress system in gSAD. Two groups with nine pair wise age and gender matched gSAD patients, who were successfully treated with the SSRI citalopram, underwent a tryptophan depletion challenge (TD) or a placebo condition. A TD procedure temporarily decreases serotonergic neurotransmission. In order to activate the stress system, the TD/placebo challenge was combined with a public speaking task. We assessed ANS responses, as measured with salivary alpha-amylase (sAA), and HPA-axis responses, as measured with salivary cortisol, both in the TD and placebo condition. The TD group showed a significantly larger sAA response as compared to the placebo group, reflecting hyperresponsivity of the ANS in this group, whereas no differences were seen in cortisol responses. This suggests that in gSAD there is a vulnerability of the ANS and not the HPA-axis. As this was found after TD, the serotonergic system may act as a mediator.

Chapter 7 is the first study that aimed to explore the influence of female reproductive hormones on gSAD symptoms. We recruited female patients with gSAD who had previously participated in our research projects in the University Medical Center Utrecht and the Leiden University Medical Center. A self-report survey with questions on the influence of menarche, the periods of the menstrual cycle, oral contraceptive use, pregnancy, lactation, postpartum period and menopause on gSAD symptoms, was returned by 46% of 140 women suffering from gSAD. Most respondents reported no influence. However, in the subgroup that did report such influences, statistical differences were found for the menstrual cycle and pregnancy. In the premenstrual period, these patients reported more severe gSAD symptoms. During pregnancy symptoms decreased, but postpartum symptom severity returned to the same levels as before pregnancy. In conclusion, a subgroup of women with gSAD seemed vulnerable for the influences of gonadal hormones. Prospective research in women with gSAD, in which the gonadal hormones are assessed, is warranted.

Thus, in general it can be concluded that our studies showed evidence for an involvement of the serotonergic, dopaminergic and noradrenergic system in gSAD. In addition, we found evidence for the involvement of the ANS as well. No evidence was found for a role of the HPA-axis. In a subgroup of patients the female gonadal hormones may influence the course of gSAD.

Discussion

In this thesis, we report on our investigations regarding the involvement of several neurotransmitter and hormonal systems in gSAD. In this discussion we first will present a neurobiological model for gSAD as proposed by Tillfors (2004). This model is the most extensive model for gSAD thus far. We will discuss the meaning of our research findings in the context of this model. Finally we will discuss limitations of this thesis and suggest future directions for research in gSAD.
Neurobiological model for gSAD by Maria Tillfors (2004)

Tillfors describes family, twin and genetic studies that underscore the heritability of gSAD, although genetic factors do not nearly explain all the variance. She states that the strongest evidence comes from the twin studies of Kendler and colleagues, which showed that in women genetic factors explain one third of the variance and in men a quarter of the variance (Kendler et al., 1992; Kendler et al., 2001). Genetic influences have also been found for temperamental traits that are thought to be related to the development of gSAD, such as behavioural inhibition, neuroticism, introversion and harm avoidance. For example, behavioural inhibition is a temperamental construct that is suggested to partly have a genetic basis. Physiological correlates of behavioural inhibition include sympathetic hyperactivity, which is interpreted as being associated with a lower threshold of excitability of the amygdala. Behavioural inhibition in children is thought to be a precursor for anxiety disorders in general and gSAD in particular later in life.

There is evidence that the amygdala, in interaction with the prefrontal cortex and the hippocampus, is crucial in the neural circuit of anxiety (Ledoux, 1998). The amygdala monitor internal and external stimuli and mediate behaviours that facilitate survival. In human research it was found that the amygdala respond to stimuli that predict threat and are involved in mediating fear/anxiety states. In gSAD exaggerated amygdala activation has been the most consistent functional neuroimaging finding (Talarovicova et al., 2007; Birbaumer et al., 1998; Lorberbaum et al., 2004; Tillfors et al., 2001; Stein et al., 2002; Shah et al., 2009). After successful treatment for gSAD, amygdala activation was decreased during public speaking (Furmark et al., 2002). The fast thalamus-amygdala pathway is suggested to provide the amygdala with information of the external world, in order to allow a person to respond to stimuli immediately. The slower thalamo-cortical-amygdala pathway provides the amygdala with information on objects and events (Ledoux, 1998). Visceral afferent information ascents from the brainstem and the hypothalamus. The reciprocal interaction with the hippocampus and related regions, allows the amygdala to incorporate information such as contextual analyses as well as an individual's prior experience (memory). This in turn will allow emotions to be triggered by fearful memories. Finally, input from the ventromedial prefrontal cortex is suggested to modulate emotional reactivity via inhibitory influences on the amygdala. The amygdala mediate autonomic, neuroendocrine, and skeletal motor responses subserving different expressions of anxiety and fear. Projections to the striatum are thought to control avoidance and approach behaviour. The amygdala coordinate the actions of the autonomic and endocrine systems by means of direct connections to the hypothalamus (Talarovicova et al., 2007). Projections to the lateral hypothalamus (via the brainstem) control sympathetic nervous system activation and projections to the paraventricular hypothalamus and the bed nucleus of the stria terminalis control the release of neuroendocrine hormones such as gonadal and adrenocorticol hormones. Amygdaloid neurons project directly to the modulatory cholinergic, dopaminergic, noradrenergic, and serotonergic systems (Talarovicova et al., 2007; Tillfors, 2004; Rodrigues et al., 2009).
Serotonin

Serotonin is a wide range regulatory neurotransmitter that is present in the brain and other parts of the body. It has an important role in the amygdala-based fear conditioning pathways, which is described in the above section. Thus, modulation of the serotonergic system influences noradrenergic activity, the release of CRH and modifies defense/escape behaviours (Stein et al., 2002). Personality traits related to anxiety are associated with the serotonergic system as is shown in research on the meaning of polymorphisms in the serotonin transporter gene regulatory region (5-HTTLPR), which has two variants. The short allele is associated with neuroticism and harm avoidance (Lesch et al., 1996). Other findings are that human subjects with one or two copies of the short allele exhibit greater amygdala neuronal activity as measured with fMRI responses to pictures of frightened or angry faces (Hariri et al., 2002). Furthermore, subjects with a short allele show a stronger coupling between amygdala and prefrontal cortex fMRI responses to aversive pictures. The prefrontal cortex can act to suppress the amygdaloid output (Heinz et al., 2005). The presence of the short allele in gSAD patients was associated with elevated trait anxiety and depression scores, with a tendency also for neuroticism, and with enhanced excitability of the right amygdala when speaking in public (Furmark et al., 2004). Nevertheless, no genetic linkage to the serotonin transporter was found in gSAD (Stein et al., 1998). This may indicate a modifying role of 5-HTTLPR polymorphisms in gSAD. Apart form studies on the genetics of gSAD, also medication, challenge and neuroimaging studies have been performed in gSAD. The efficacy of selective serotonin reuptake inhibitors (SSRIs), the results of serotonergic challenges and the finding of reduced binding of the 5HT1A-receptor in the amygdala also suggest the involvement of the serotonergic system in gSAD (Ipser et al., 2008; Hollander et al., 1998; Tancer et al., 1994; Lanzenberger et al., 2007).

Results of this thesis

Our studies added evidence for the involvement of the serotonergic system in gSAD and make the hypothesis of Tilfors (2004) on the role of altered serotonergic function in gSAD more specific. The results of our challenge study with meta-chlorophenylpiperazine (m-CPP) (chapter 2) might reflect hypersensitivity of the 5HT2C-receptor in gSAD compared to controls and furthermore show that panic disorder and gSAD are distinct psychiatric disorders on a neurobiological level. The hypersensitivity of this receptor might be the result of decreased serotonin activity. We also found increased binding patterns to the serotonin transporter in the thalamus in gSAD with a 123I-β-(4-iodophenyl)-tropane SPECT scan. This might be the result of decreased extracellular serotonin levels near the transporter, allowing 123I-β-CIT to bind with higher density or perhaps the increased binding to the serotonin transporter reflects higher numbers of the serotonin transporter in the thalamus and results in a lowering of extracellular serotonin.

Based on our study results we hypothesize that gSAD is associated with decreased serotonin activity, which also is concordant with studies indicating that SSRIs are effective in the treatment of gSAD. According to the theory of Tilfors, decreased serotonergic control probably also influences the stress system. Based on the results of our studies we hypothesize that in gSAD
decreased serotonergic control of the stress system leads to an increased stress response. The involvement of the thalamus as we reported in our study, suggests alterations in the perception of the external world in gSAD, and probably is associated with the experience of the external world as more threatening and scrutinizing.

Dopamine
Research has been done on the dopamine D₄-receptor gene and the temperamental trait novelty seeking, which is a central feature of behavioural inhibition. An association was found between the longer allele of polymorphic exon III repeat sequences of the D₄-receptor and high levels of novelty seeking, probably reflecting decreased receptor sensitivity (Ebstein et al., 1996; Benjamin et al., 1996; Ebstein et al., 1997; Noble et al., 1998; Ono et al., 1997; Strobel et al., 1999). Low extraversion (~intraversion) was associated with single nucleotide polymorphisms (SNPs) within the COMT gene. Furthermore, Rowe et al. reported an association between a polymorphism in the dopamine transporter gene and SAD in children (Rowe et al., 1998).

Li (2008) described that the brain regions that seem to be involved in gSAD, such as the amygdala, thalamus, prefrontal cortex and striatum, are densely innervated not only by serotonergic but also by dopaminergic neurons. In this article the hypothesis was formulated that impaired striatal-thalamic filtering of information relevant for social evaluation and an excessive conditionability of striatal-amygdalal circuits may play a central role in the pathophysiology of gSAD.

Several studies indicated that dopamine is involved in the neurobiology of gSAD, as was described in chapter 3 of this thesis in more detail. The involvement of the dopaminergic system in gSAD was first suggested by the increased prevalence of gSAD in Parkinson’s disease (Stein et al., 1990). Parkinson’s disease is characterized by striatal dopaminergic hypofunction. Not only was the prevalence of gSAD very high (50%) in Parkinson’s disease, it also appeared that anxiety symptoms precede the symptoms of Parkinson’s disease (Kummer et al., 2008). Another study showed a lower level of homovanillic acid, a metabolite of dopamine, in the cerebrospinal fluid (Johnson et al., 1994). Also the efficacy of MAOIs is compatible with the involvement of the dopaminergic system. Direct evidence of the dopaminergic involvement in gSAD has been shown in a few neuroimaging studies, in which decreased binding of the dopamine transporter and decreased binding of the D₂-receptor was found (Tiihonen et al., 1997; Schneier et al., 2000).

Results of this thesis
The results of our study with ¹²³I-β-CIT SPECT in psychotropic medication-naïve patients with gSAD also supports the role of the dopaminergic system, as we found a significantly higher binding potential for the dopamine transporter in the striatum. In other papers such a relationship could not be demonstrated. Tiihonen and colleagues (1997) and, recently, Schneier et al. (2009), published opposite findings. However, the study of Tiihonen et al. suffered from some methodological shortcomings as was explained in chapter 3 (Tiihonen et al., 1997). In the study of Schneier et al., the authors tried to unravel the dopaminergic involvement in gSAD by studying D₂-receptor availability, dopamine release, and dopamine transporter availability by using PET
and SPECT scans (Schneier et al., 2000). Apart from the fact that they did not exclusively include psychototropic medication-naïve patients, which they mentioned in their discussion, they also did not exclude patients with psychiatric comorbidity. Furthermore, they group wise matched on age and gender in stead of the pair wise matching we did. With respect to the dopamine transporter availability in the striatum our study is the one with the least confounders.

The increased binding we found of $^{123}$I-β-CIT to the dopamine transporter in the striatum might be the result of decreased extracellular dopamine levels near the transporter, allowing $^{123}$I-β-CIT to bind with higher density, or the lower dopamine levels to be the result of higher numbers of dopamine transporters in the striatum. This might be in accordance with the reduced neural activation in the striatum that was found in a study in which gSAD patients and controls underwent a fMRI while performing a implicit sequence learning task (Sareen et al., 2007).

Our study confirms the theory of Tillfors (Tillfors, 2004), which states that the dopaminergic system is involved in the neurobiology of gSAD. Combining the results of the above described studies, we hypothesize a decreased dopaminergic functioning in gSAD, which is concordant with the increased prevalence of gSAD in Parkinson’s disease. This probably is also compatible with the increased prevalence of alcoholism in patients with gSAD, since the decreased activity of dopamine as a result of long term use of alcohol is seen as a maintaining factor in alcoholism. The acute administration of alcohol leads to increased dopaminergic neurotransmission (Soderpalm et al., 2009). According to the model of Tillfors, the involvement of the striatum might be associated with the avoidance of social situations, which is a feature of gSAD.

**Stress system**

The stress system involves the hypothalamic-pituitary-adrenal axis (HPA-as) and the Autonomic Nervous System (ANS). Stress initiates the release of corticotrophin releasing hormone (CRH), which potentiates the stress response by organizing the ANS response and the HPA-axis response. Thus both branches of the stress system are activated in times of stress. Both branches have been studied in gSAD, albeit separately and not in the same study.

**Stress system: Hypothalamic Pituitary Adrenal Axis (HPA-axis)**

Research on the HPA-axis in gSAD showed that thus far, no HPA-axis dysfunctions in basal non-stress, conditions could be found, as measured with 24h-urine, diurnal salivary cortisol curves and urinary cortisol after dexamethasone suppression (Potts et al., 1991; Uhde et al., 1994; Martel et al., 1999). However, some studies, but not all, reported increased cortisol responses to stress (Martel et al., 1999; Condren et al., 2002; Furlan et al., 2001; Roelofs et al., 2005; Roelofs et al., 2009). Roelofs et al. also found that increased cortisol responses were significantly correlated to the increase in social avoidance behaviour as measured by the approach-avoidance task (Roelofs et al., 2009).

**Results of this thesis**

In the study described in chapter 4 we found no evidence for dysfunctions of the HPA-axis, during the cortisol awakening rise (CAR), in ‘basal’ late afternoon levels, nor during dexamethasone
suppression, based on cortisol saliva assessments. However, other studies described above showed a hyperreactive HPA-axis in response to a psychosocial challenge. So it might be that HPA-axis abnormalities in gSAD only occur in situations related to a stressor for which sufferers of gSAD are vulnerable.

**Stress system: Autonomic Nervous System**

The involvement of the noradrenergic system and the ANS is to be expected if one observes the symptoms of gSAD. The efficacy of serotonin-noradrenalin reuptake inhibitors (SNRIs) implies that noradrenergic dysfunction is associated with gSAD. Previous studies on the noradrenergic system and the ANS in gSAD were done by for example measuring plasma noradrenaline levels, heart rate, and blood pressure. (Stein et al., 1994; Grossman et al, 2001; Gerlach et al., 2003; Laederach-Hofmann et al, 2002). Abnormalities found in one study often could not be replicated in another (Ipser et al., 2008; Stein et al., 1992; Bouwer and Stein, 1998). However, major drawback in all these studies was the stress accompanying the sampling procedure (e.g. venipuncture) and the autonomic measures such as heart rate and blood pressure that are easily influenced by many factors (e.g. posture). These factors may well be responsible for the contradictory results.

**Results of this thesis**

In chapter 4 we described a pilot study with mirtazapine 30 mg, which was effective in 41.7% of the patients. These results might indicate that dysregulations of the noradrenergic and serotonergic system in gSAD are the substrate for the efficacy of mirtazapine.

In chapter 5 we describe a study in which we investigated the ANS function in gSAD in basal non-stressed conditions. We investigated the sAA awakening response, the diurnal curve and a low dose (0.5 mg) dexamethasone suppression test in gSAD. Even though we sampled in non-stressed conditions, sAA levels were almost twice as high in gSAD patients as compared to the control subjects. This is a remarkable finding even more so because it shows the imbalance between the HPA-axis and ANS in gSAD.

**Gonadal hormones**

There are no studies that report on the influence of female gonadal hormones on gSAD symptoms as yet. Since the onset is often at puberty, and more women are affected and more severely than men, it might be expected that these hormones have an influence on gSAD.

**Results of this thesis**

In chapter 7 we report on the results of our retrospective inventarisation of the influences of female hormonal phases on social anxiety and avoidance in female gSAD patients. Most women did not report any influence of the hormonal phases on gSAD symptoms. A subgroup of women however, reported that gSAD symptoms increased premenstrually and decreased during pregnancy. This possibly might indicate that a subgroup of gSAD women is vulnerable for the influences of gonadal hormones on symptom severity.
Limitations

In this thesis we explored the role of several hormonal and neurotransmitter systems likely to be involved in gSAD. Just because of this explorative approach the study of each of the systems remained rather superficial. This is a limitation of this thesis as it restricts the possibility to draw definitive conclusions. However, we think this approach is justified because, as we discussed already in the introduction, the neurobiology of gSAD is largely a terra incognita. Most limitations of the separate studies were already discussed in the corresponding chapters. We will not repeat them here. However, some additional remarks must be make in this final chapter. In chapter 2 we describe a m-CPP challenge. As we know now, a m-CPP challenge is not a pure serotonergic challenge, which make the results more difficult to interpret. It is known to be a (partial) 5-HT$_{2c}$ receptor agonist that also possesses moderate to low affinity for other 5-HT receptors, as well as for (α$_2$) adrenergic and dopamine receptors. Therefore the effects of m-CPP could also partly be the effect of the influence on the adrenergic or dopaminergic receptors. In chapter 4 we did a mirtazapine pilot study. This study was not blinded and not placebo-controlled. Therefore these results are preliminary. We cannot rule out the possibility that the effects of mirtazapine in this study are predominantly a placebo effect. The results have to be replicated in a double blind, placebo-controlled trial in order to draw more definite conclusions. In chapter 5 and 6 we studied HPA-axis functioning. We took the circadian rhythm of cortisol in account, but not the pursatil release of cortisol, which could have influenced our results. Cortisol binds for 95% to large proteins, mostly cortisol binding globulin (CBG) and albumine. Only the 5% free cortisol is thought to be biologically active. Free cortisol enters saliva by passive diffusion, which means that the fraction of free cortisol is measurable in saliva. Thus a limitation is that in our studies we only measured the fraction of free cortisol in saliva which accurately reflects the free cortisol fraction in the blood, but does not reflect the total cortisol levels in the blood. In chapter 6 we used a public speaking challenge combined with a tryptophan depletion test. With the public speaking challenge we meant to evoke specifically social anxiety. However, it might be the case that this is not the best challenge to evoke social anxiety, as was shown in a tryptophan depletion study in which social anxiety was more severe during the reading of an autobiographical script than during a public speaking challenge (Argyropoulos et al., 2004).
Conclusions

In this thesis we found evidence of the involvement of serotonin, dopamine, and noradrenaline/ANS, but not the HPA-axis, in the neurobiology of gSAD. We hypothesize that serotonin and dopamine function is decreased in gSAD, that there is hyperfunctioning of the ANS, and that HPA-axis function is not concordant with the ANS activation, as we saw in basal conditions, and in stress conditions following manipulation of the serotonergic system. We also think that there are indications that the female gonadal hormones also have a modulatory role in gSAD in a subgroup of women. This exploration of the neurobiology of gSAD leads to the conclusion that a variety of brain systems are involved in gSAD in a complex way. These results expand the model as was proposed by M. Tillfors in 2004.

With the exception of the HPA-axis and the ANS, we did not study the way in which the neurotransmitter (serotonin, dopamine and noradrenaline) systems we studied interact in gSAD. However, several studies report on the interaction between these systems, although not in gSAD. The influence of serotonin on the HPA-axis has been studied several times in depression, showing that under conditions of chronically elevated corticosteroid concentrations, serotonergic neurotransmission is impaired (van Praag, 1996; Meijer and De Kloet, 1998). Polymorphisms of the serotonin transporter affected the HPA-axis stress response to painful stimulation in newborn babies (Mueller et al., 2009). In a review of Lanfumey the reciprocal interactions between the HPA-axis and the serotonergic system are well described. The hippocampus seems to play a particular role in the serotonergic-HPA-axis interactions in response to stress (for a review see Lanfumey et al., 2008). The interplay between noradrenaline and serotonin depends on the region and receptors that are activated as was described by Demling et al. (2009). Animal research has shown that manipulation of the serotonergic system leads to autonomic dysregulation. In mice with excessive serotonergic
autoinhibition was found that they were not able to activate autonomic target organs in response to environmental challenges (Audero et al., 2008). In mice lacking serotonin reduced heart rate and respiration was found (Alenina et al., 2009). In chapter 5 we described a tryptophan depletion study, combined with a public speaking challenge, in gSAD patients who were responders to SSRI-treatment. In this study two groups of gSAD patients were tested, one group underwent the tryptophan depletion, the other group a control condition. In this study no differences were seen in cortisol responses following the public speaking challenge after serotonergic manipulation. Importantly, we did find hyperresponsiveness of the ANS to stress after serotonergic manipulation in medicated gSAD patients.

These studies show that the two branches of the stress system do not act in concert in gSAD, both in basal and in stress conditions, which is a remarkable finding. The results are in line with the hyperarousal that is part of the phenomenology in gSAD. Therefore ANS hyperfunction may play an important role in the neurobiology of gSAD, although the specificity of these results for gSAD has yet to be determined. Our finding of basal hyperfunction of the ANS confirms the hypothesis of Tillfors (2004) who postulated that the hyperfunction could be associated with a lower threshold of excitability of the amygdala. These findings might also have implications for the efficacy of pharmacological agents. As yet, no clinical trials are published that compare the efficacy of SSRIs with agents that also influence the noradrenergic system.

**Future research**

Future research may focus on the specificity of the imbalance between the HPA-axis and the ANS system in gSAD. Therefore the HPA-axis and ANS should be studied in concert in other psychiatric disorders. In addition, we think that the dopaminergic system should be further investigated as well as the interactions between the several brain systems. It would be of interest to also study these systems in children that suffer from gSAD, or are at risk for the development of gSAD, in order to get more insight in the imbalances at the moment that the illness becomes manifest, before all kinds of compensatory mechanisms of the brain have become active. Finally, our suggestion for a next step in pharmacological research in gSAD is to investigate agents that influence predominantly autonomic function and compare them with SSRIs.
References


