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

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THE EMBARRASSED BRAIN

Towards a neurobiology of generalized social anxiety disorder

Frederieke van Veen

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THE EMBARRASSED BRAIN

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THE EMBARRASSED BRAIN

Towards a neurobiology of generalized social anxiety disorder

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Voor Rachel en Sofie

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Introduction

Introduction

Since the 1980s, social anxiety disorder (SAD) was recognized as a diagnostic entity (American Psychiatric Association, 1980). As described in DSM-IV-TR, social anxiety disorder 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. Examples of such situations are meetings, parties, speaking in front of an audience, or making a phone call in public. The feared situations are avoided or are endured with intense anxiety and distress (American Psychiatric Association, 2000). Experiencing anticipatory anxiety and shame afterwards are characteristic of SAD. Somatic symptoms of anxiety that occur in social situations are palpitations, blushing, trembling and sweating. Two subtypes of SAD can be distinguished: the specific (sSAD) and the generalized type (gSAD) (American Psychiatric Association, 2000), the latter being the most disabling, most severe and complete subtype, showing all aspects of social anxiety. In this thesis, we chose to investigate gSAD, anticipating to find most pronounced neurobiological dysfunctions.

SAD is among the most prevalent mental disorders, however, most epidemiological studies did not make a distinction between subtypes. Reason for this is that at first there was no evidence for a possible distinction, and DSM-III defined social phobia primarily as performance anxiety. In DSM-III-R (1987) the generalized subtype was introduced, in which the phobic situation included most social situations, but no additional criteria were provided. In 1995, Manuzza et al. showed that generalized and non-generalized social phobia were valid subtypes, and that on a biological level familial social phobia was more common among patients with generalized social phobia (Mannuzza et al., 1995).

In European community studies, lifetime prevalence rates of 3.9 to 13.7% according to DSM-IV criteria were found and more women were affected than men (Fehm et al., 2005). The National Comorbidity Survey Replication in the United States reported life time prevalence rates of 12.1% (Ruscio et al., 2008). An epidemiological survey in Ontario, Canada, did discriminate between the specific (sSAD) and the generalized subtype (gSAD) and reported life time prevalence rates of 7.0% for sSAD and 5.9% for gSAD (Stein and Kean, 2000). The prevalence estimates of gSAD were higher in women than in men, but no exact data were reported on this (Stein and Kean, 2000). Symptoms of gSAD might also be more severe in women than in men. The onset of gSAD is often at puberty or before (Keller, 2003; Wittchen and Fehm, 2003; Stein and Kean, 2000).

gSAD is still generally underrecognized even among psychiatrists and the effects of gSAD are still underestimated. The National Comorbidity Survey showed that gSAD is associated with impairment of social functioning, family-life and close relationships (Lampe et al., 2003; Patel et al., 2002; Stein and Kean, 2000; Wittchen et al., 1999). In addition, patients with gSAD are less likely to be in a relationship or marriage (Dingemans et al., 2001). gSAD was also associated with early leave of school (Stein and Kean, 2000), lower level of education (Katzelnick and Greist, 2001; Wittchen et al., 1999), a higher risk of being unemployed (Patel et al., 2002; Lampe et al., 2003), and engagement in jobs below the level of qualification (Katzelnick and Greist, 2001). Furthermore, shame leads to patients delay in receiving treatment (Dingemans et al., 2001). gSAD patients

without comorbid disorders but with the worst fears were least likely to receive treatment (Ruscio et al., 2008).

Although gSAD is a disabling disease, only half of the patients receive treatment (Ruscio et al., 2008). Treatments of choice for gSAD are serotonin reuptake inhibitors (SSRIs), serotonin-noradrenalin reuptake inhibitors (SNRIs) and cognitive behavioural therapy (CBT) (Bandelow et al., 2007; Ipser et al., 2008). Monoamine oxidase inhibitors (MAOIs) and benzodiazepines are also effective, but are regarded to be second-line agents: MAOIs, because treatment requires dietary and medication restrictions, and benzodiazepines, because of cognitive adverse events, addiction and the requirement of slow withdrawal (Ipser et al., 2008).

Neurobiological background

The underpinnings of the neurobiology of gSAD are not clear yet. Research in other affective disorders showed that several hormonal and neurotransmitter systems such as the serotonergic and dopaminergic neurotransmitter systems, are involved in the neurobiology of these disorders, as well as the stress system and female gonadal hormones, as will be described in short in the following section.

The efficacy of SSRIs suggests that the neurotransmitter serotonin, regulating among other things mood and anxiety, might be involved in major depressive disorder, posttraumatic stress disorder (PTSD), generalized anxiety disorder (GAD) and panic disorder (PD) (Hoffman and Mathew, 2008; Vaswani et al., 2003). Dopamine, central in reward and motivation, might be involved in the neurobiology of PTSD, as was shown in a study in which increased excretion of dopamine and its metabolite were found in the urine of PTSD patients (Heim and Nemeroff, 2009). In addition, dysregulation of the stress system, the hypothalamic-pituitary-adrenal-axis (HPA-axis) and autonomic nervous system (ANS), in affective disorders was reported. Cortisol, the major final product of the HPA-axis in humans, modulates at several levels the function of many neurotransmitters, including serotonin and dopamine. Hyperfunctioning of the HPA-axis was found in major depression, whereas in PTSD predominantly hypofunctioning was found with increased sensitivity of the HPA-axis to negative glucocorticoid feedback (Swaab et al., 2005; Brown et al., 2009; Heim and Nemeroff, 2009). Furthermore, in panic disorder, HPA-axis hyperactivity in response to contextual cues was reported (Abelson et al., 2007). Other studies found that baseline HPA-axis functioning in panic disorder was the same as in healthy controls (Strohle and Holsboer, 2003). Hyperfunctioning of the HPA-axis was also reported in generalized anxiety disorder (GAD) (Mantella et al., 2008). Hyperactivity of the other branch of the stress system, the autonomic/sympathetic nervous system, was described in major depression, PTSD, and might be the case in PD (Brown et al., 2009; Heim and Nemeroff, 2009; Grassi and Kiowski, 2002). Studies on female gonadal hormones reported that they affect the course and severity of several symptoms of anxiety. Premenstrual increase of anxiety symptoms was reported in healthy women, and exacerbation of psychiatric symptoms in women with depressive disorder and in women with anxiety disorders, such as GAD and PD (Rofe et al., 1993; Hsiao et al., 2004; Brambilla et al., 2003).

Aim of the thesis

Knowledge of the neurobiology of gSAD is essential for the development of new treatments. Since many systems seem to be involved in the neurobiology of affective disorders, we aimed in this thesis to make an exploration of the serotonergic and dopaminergic systems, the two branches of the stress system, namely the HPA-axis and the noradrenergic system/ANS, and we did a first step in studying the influence of reproductive hormones in gSAD.

Introduction of the neurotransmitter and hormonal systems that were studied in this thesis

Serotonin (5-hydroxytryptamine = 5-HT) is a metabolite of the amino acid tryptophan. Following transport of tryptophan into the serotonin neuron, tryptophan is converted into 5-hydroxytryptophan (5HTP) by the enzyme tryptophan hydroxylase, which is the rate limiting step in the synthesis. 5HTP is then quickly converted into serotonin by the enzyme aromatic amino acid decarboxylase. After release into the synapse, the serotonin transporter regulates the availability of serotonin in the synapse by a reuptake mechanism. Serotonin is a regulatory neurotransmitter and is among other functions involved in the regulation of stress, mood, sleep, appetite, impulse control, and reproduction.

Dopamine is a catecholamine synthesized from the precursor tyrosine. The activities of tyrosine hydroxylase, the rate limiting step, and dihydroxyphenylalanine decarboxylase lead to production of dopamine. Dopamine can be metabolized by catechol-O-methyltransferase (COMT) or monoamine oxydase (MAO), the same enzymes involved in the metabolism of norepinephrine and epinephrine. Dopamine is thought to be involved in motivation, reward, reinforcement and motor functions and plays a poorly understood role in some sympathetic ganglia of the ANS.

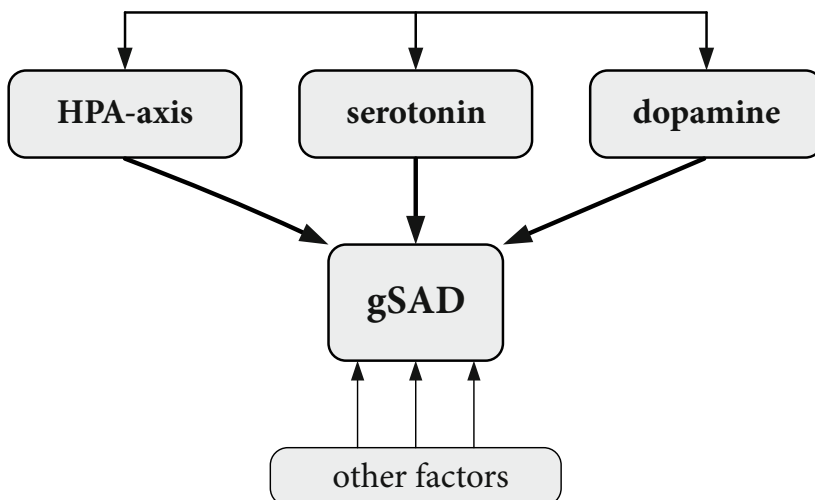
Central in stress response is regulation of the hypothalamic-pituitary-adrenal axis (HPA-axis) and the ANS. Stress initiates the release of corticotrophin releasing hormone (CRH), which potentiates the stress response by organizing the ANS response and the HPA-axis response. Thus both branches of the stress system are activated in times of stress, and therefore are likely to be hyperactive in anxiety disorders.

HPA-axis activity is regulated by CRH and arginine-vasopressine (AVP), which are released in the paraventricular nucleus of the hypothalamus. They coordinate the release of adrenocorticotrope hormone (ACTH) by the pituitary. ACTH induces the secretion and release of cortisol in a pulsatile manner from the adrenal glands. Cortisol modulates at the periphery energy mobilization, the immune system, bone and muscle growth, epithelial cell growth, erythroid cell production and the cardiovascular system. In the brain cortisol influences the limbic system by binding to two receptors, the high affinity mineralocorticoid receptor (MR) and the low affinity glucocorticoid receptor (GR). The GR plays an important role in the negative feedback of the system. HPA-axis activity is determined by two factors: stress and the normal circadian rhythm (Lanfumey et al., 2008). Recent studies in healthy humans indicated that HPA-axis function in stress and in non-stressed conditions is highly complex controlled both by limbic structures, including the amygdala and the hippocampus

(Buchanan et al., 2004; Kern et al., 2008) and the prefrontal cortex (Kern et al., 2008).

The somatic symptoms of gSAD like palpitations, blushing, trembling and sweating are under autonomic control. Noradrenaline is synthesized from the precursor tyrosine. In the periphery, the most prominent neurons that synthesize noradrenaline are the sympathetic ganglion cells of the Autonomic Nervous System (ANS). In the brain, noradrenaline is produced in the locus coeruleus, a brainstem nucleus that projects to forebrain targets, influencing sleep and wakefulness, attention, and feeding behaviour. The reciprocal interaction between the locus coeruleus and the paraventricular nucleus provides a link between both systems. One of the important receptors is the α_2 -adrenergic autoreceptor, modulating presynaptically the release of several other neurotransmitters. The ANS is influenced by many brain structures, mostly via the hypothalamus. The hypothalamus integrates all the information into a coherent pattern of autonomic response. The hypothalamus regulates the ANS in two ways. It projects to neurons in the brain stem and spinal cord for the control of temperature, heart rate, blood pressure and respiration.

The gonadal hormones estrogen and progesterone regulate female hormonal phases and are also considered neuroactive steroids, because of their capacity to modify neural activities (Le Melledo et al., 2001; Dubrovsky, 2005). Estrogens influence the serotonergic system through the estrogen receptor $ER\beta$ by promoting serotonergic transmission (Osterlund et al., 2005). Progesterone interacts with several neurotransmitter systems, neuropeptides and the HPA-axis. It influences anxiety probably by its effects on the gamma-aminobutyric acid (GABA)_A-receptor. The GABA_A-receptor modulates the output of for example the dopaminergic, adrenergic, and serotonergic systems (Le Melledo and Baker, 2004). Furthermore, sex steroids play a role in lifelong structural plasticity of several brain regions, including areas involved in stress response, such as the amygdala and hippocampus (McEwen and Magarinos, 2001).



Outline of the thesis

In **chapter 2** we describe a serotonergic challenge with rapid intravenous meta-chlorophenylpiperazine (m-CPP) in seven patients with panic disorder, seven patients with gSAD and seven healthy controls in order to confirm the involvement of serotonin in gSAD and to evaluate the possibility of a shared neurobiology of gSAD and panic disorder. For this study we used meta-chlorophenylpiperazine (m-CPP), which is 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. Rapid intravenous administration of 0.1 mg/kg m-CPP is highly sensitive and selective in the provocation of panic attacks in patients with panic disorder as compared to healthy controls (Van Der Wee et al., 2004). It was our aim to confirm that serotonin is involved in the neurobiology of gSAD and that gSAD and panic disorder are neurobiologically distinct disorders.

In **chapter 3** we studied the involvement of the serotonergic and dopaminergic system in gSAD by means of a single photon emission computed tomography (SPECT) neuroimaging study with ¹²³I- β -(4-iodophenyl)-tropane (¹²³I- β -CIT), which binds to the serotonin and dopamine transporters, in twelve gSAD patients and twelve healthy controls. We used ¹²³I- β -CIT SPECT to visualize both the dopamine and the serotonin transporter in the human brain after a single administration of the ligand. Binding of ¹²³I- β -CIT in the striatal region has been shown to reflect mainly binding to the dopamine transporter, binding in the thalamus, midbrain and pons to reflect predominantly binding to the serotonin transporter (Pirker et al., 1995; De Win et al., 2005). The first SPECT scan was made four hours after the infusion of ¹²³I- β -CIT to visualize serotonin transporter binding, and another SPECT scan was made twenty-four hours after infusion to visualize dopamine transporter binding. It was our aim to find differences in the dopamine and serotonin transporter binding in gSAD.

Chapter 4 describes an open-label pilot study in which we investigated the efficacy of mirtazapine, an antidepressant blocking the α_2 -adrenergic autoreceptors and therefore stimulating noradrenergic and serotonergic pathways in gSAD. In this study fourteen gSAD patients were treated for twelve weeks with mirtazapine 30 mg. The primary outcome measure was the change in score on the Liebowitz Social Anxiety Scale (Liebowitz, 1987). We expected to find that mirtazapine might be an effective treatment in gSAD.

In **chapter 5** we studied the involvement of the stress system in gSAD in basal (non-challenging) conditions. We investigated the two branches of the stress system, the HPA-axis and the ANS, in concert in 43 gSAD patients and 43 controls in basal (non-challenging) conditions, and after a low dose of dexamethasone to investigate the feedback sensitivity. We used the non-invasive markers salivary cortisol for the HPA-axis and salivary alpha-amylase (sAA) for the ANS. sAA is a relatively new marker for the ANS. sAA is produced by the salivary glands, primarily by acinar cells. Acinar cells are innervated by both the sympathetic and parasympathetic nervous system. The results of studies in animals and humans indicate that the ANS plays a powerful role in the secretion of sAA, with contributions of both the alpha-adrenergic and beta-adrenergic mechanisms. Therefore sAA might be regarded as a marker of autonomic activation (For a review

see Nater and Rohleder, 2009). We aimed to find differences in the activation of the two branches of the stress system in basal non-stressed conditions in gSAD.

The interplay between the serotonergic system and the two branches of the stress system in gSAD was studied as described in **chapter 6**. Therefore the cortisol and sAA responses to a tryptophan depletion challenge versus a control condition combined with a public speaking challenge, were measured in two groups of nine gSAD patients. Acute tryptophan depletion is a procedure that temporarily decreases serotonergic neurotransmission (Hood et al., 2005). Drinking a large neutral amino-acid (LNAA) mixture without tryptophan (TRP) leads to a decreased plasma TRP/LNAA ratio. Since TRP and large neutral amino acids (LNAAs) compete for transport through the blood-brain-barrier, less TRP will be available in the brain, decreasing the synthesis of serotonin. This in turn diminishes the effects of SSRIs (Hood et al., 2005). It was our aim to find differences in the activation of the autonomic nervous system and the HPA-axis following stress after manipulation of the serotonergic system in gSAD.

In **chapter 7** we describe a retrospective inventory of the course of gSAD symptoms during the female hormonal cycle to investigate whether female gonadal hormones are likely to be involved in the neurobiology of gSAD. Female gSAD patients completed a self-report survey with questions regarding the menarche, menstrual cycle, oral contraceptive use, pregnancy, lactation, postpartum period and menopause. Women that did report an influence of these phases on gSAD symptoms were asked to rate the severity of gSAD symptoms in these variable hormonal phases. We aimed to find that the fluctuations of female reproductive hormones might influence symptoms of gSAD in women.

The summary, conclusions and discussion of this thesis will be presented in **chapter 8**, including the incorporation of these data in a neurobiological model of gSAD.

References

- Abelson, J. L., Khan, S., Liberzon, I., and Young, E. A. (2007). HPA axis activity in patients with panic disorder: review and synthesis of four studies. *Depress.Anxiety*, 24, 66-76.
- American Psychiatric Association (1980). *Diagnostic and Statistical Manual of Mental Disorders-DSM III*. American Psychiatric Press, Washington, DC.
- American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders DSM-IV Text Revision* American Psychiatric Press, Washington, DC.
- Bandelow, B., Seidler-Brandler, U., Becker, A., Wedekind, D., and Ruther, E. (2007). Meta-analysis of randomized controlled comparisons of psychopharmacological and psychological treatments for anxiety disorders. *World J.Biol.Psychiatry*, 8, 175-187.
- Brambilla, F., Biggio, G., Pisu, M. G., Bellodi, L., Perna, G., Bogdanovich-Djukic, V. et al. (2003). Neurosteroid secretion in panic disorder. *Psychiatry Res.*, 118, 107-116.
- Brown, A. D., Barton, D. A., and Lambert, G. W. (2009). Cardiovascular abnormalities in patients with major depressive disorder: autonomic mechanisms and implications for treatment. *CNS.Drugs*, 23, 583-602.
- Buchanan, T. W., Kern, S., Allen, J. S., Tranel, D., and Kirschbaum, C. (2004). Circadian regulation of cortisol after hippocampal damage in humans. *Biol.Psychiatry*, 56, 651-656.
- De Win, M. M., Habraken, J. B., Reneman, L., van den, B. W., den Heeten, G. J., and Booij, J. (2005). Validation of [(123)I]beta-CIT SPECT to assess serotonin transporters in vivo in humans: a double-blind, placebo-controlled, crossover study with the selective serotonin reuptake inhibitor citalopram. *Neuropsychopharmacology*, 30, 996-1005.
- Dingemans, A. E., van Vliet, I. M., Couvee, J., and Westenberg, H. G. (2001). Characteristics of patients with social phobia and their treatment in specialized clinics for anxiety disorders in the Netherlands. *J.Affect.Disord.*, 65, 123-129.
- Dubrovsky, B. O. (2005). Steroids, neuroactive steroids and neurosteroids in psychopathology. *Prog.Neuropsychopharmacol.Biol.Psychiatry*, 29, 169-192.
- Fehm, L., Pelissolo, A., Furmark, T., and Wittchen, H. U. (2005). Size and burden of social phobia in Europe. *Eur.Neuropsychopharmacol.*, 15, 453-462.
- Grassi, G. and Kiowski, W. (2002). Is the autonomic dysfunction the missing link between panic disorder, hypertension and cardiovascular disease? *J.Hypertens.*, 20, 2347-2349.
- Heim, C. and Nemeroff, C. B. (2009). Neurobiology of posttraumatic stress disorder. *CNS.Spectr.*, 14, 13-24.
- Hoffman, E. J. and Mathew, S. J. (2008). Anxiety disorders: a comprehensive review of pharmacotherapies. *Mt.Sinai J.Med.*, 75, 248-262.
- Hood, S. D., Bell, C. J., and Nutt, D. J. (2005). Acute tryptophan depletion. Part I: rationale and methodology. *Aust.N.Z.J.Psychiatry*, 39, 558-564.
- Hsiao, M. C., Hsiao, C. C., and Liu, C. Y. (2004). Premenstrual symptoms and premenstrual exacerbation in patients with psychiatric disorders. *Psychiatry Clin.Neurosci.*, 58, 186-190.
- Ipser, J. C., Kariuki, C. M., and Stein, D. J. (2008). Pharmacotherapy for social anxiety disorder: a systematic review. *Expert.Rev.Neurother.*, 8, 235-257.

- Katzelnick, D. J. and Greist, J. H. (2001). Social anxiety disorder: an unrecognized problem in primary care. *J.Clin.Psychiatry*, 62 Suppl 1, 11-15.
- Keller, M. B. (2003). The lifelong course of social anxiety disorder: a clinical perspective. *Acta Psychiatr. Scand.Suppl*, 85-94.
- Kern, S., Oakes, T. R., Stone, C. K., McAuliff, E. M., Kirschbaum, C., and Davidson, R. J. (2008). Glucose metabolic changes in the prefrontal cortex are associated with HPA axis response to a psychosocial stressor. *Psychoneuroendocrinology*, 33, 517-529.
- Lampe, L., Slade, T., Issakidis, C., and Andrews, G. (2003). Social phobia in the Australian National Survey of Mental Health and Well-Being (NSMHWB). *Psychol.Med.*, 33, 637-646.
- Lanfume, L., Mongeau, R., Cohen-Salmon, C., and Hamon, M. (2008). Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. *Neurosci.Biobehav.Rev.*, 32, 1174-1184.
- Le Melleo, J., Jhangri, G. S., Lott, P., Tait, G. R., McManus, K., Geddes, M. et al. (2001). Effect of medroxyprogesterone pretreatment on pentagastrin-induced panic symptoms in females with panic disorder. *Psychiatry Res.*, 101, 237-242.
- Le Melleo, J. M. and Baker, G. (2004). Role of progesterone and other neuroactive steroids in anxiety disorders. *Expert.Rev.Neurother.*, 4, 851-860.
- Liebowitz, M. R. (1987). Social phobia. *Mod.Probl.Pharmacopsychiatry*, 22:141-73., 141-173.
- Mannuzza, S., Schneier, F. R., Chapman, T. F., Liebowitz, M. R., Klein, D. F., and Fyer, A. J. (1995). Generalized social phobia. Reliability and validity. *Arch.Gen.Psychiatry*, 52, 230-237.
- Mantella, R. C., Butters, M. A., Amico, J. A., Mazumdar, S., Rollman, B. L., Begley, A. E. et al. (2008). Salivary cortisol is associated with diagnosis and severity of late-life generalized anxiety disorder. *Psychoneuroendocrinology*, 33, 773-781.
- McEwen, B. S. and Magarinos, A. M. (2001). Stress and hippocampal plasticity: implications for the pathophysiology of affective disorders. *Hum.Psychopharmacol.*, 16, S7-S19.
- Nater, U. M. and Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology*, 34, 486-496.
- Osterlund, M. K., Witt, M. R., and Gustafsson, J. A. (2005). Estrogen action in mood and neurodegenerative disorders: estrogenic compounds with selective properties-the next generation of therapeutics. *Endocrine.*, 28, 235-242.
- Patel, A., Knapp, M., Henderson, J., and Baldwin, D. (2002). The economic consequences of social phobia. *J.Affect.Disord.*, 68, 221-233.
- Pirker, W., Asenbaum, S., Kasper, S., Walter, H., Angelberger, P., Koch, G. et al. (1995). beta-CIT SPECT demonstrates blockade of 5HT-uptake sites by citalopram in the human brain in vivo. *J Neural Transm.Gen Sect.*, 100, 247-256.
- Rofe, Y., Blittner, M., and Lewin, I. (1993). Emotional experiences during the three trimesters of pregnancy. *J.Clin.Psychol.*, 49, 3-12.
- Ruscio, A. M., Brown, T. A., Chiu, W. T., Sareen, J., Stein, M. B., and Kessler, R. C. (2008). Social fears and social phobia in the USA: results from the National Comorbidity Survey Replication. *Psychol. Med.*, 38, 15-28.

-
- Stein, M. B. and Kean, Y. M. (2000). Disability and quality of life in social phobia: epidemiologic findings. *Am.J.Psychiatry*, 157, 1606-1613.
- Strohle, A. and Holsboer, F. (2003). Stress responsive neurohormones in depression and anxiety. *Pharmacopsychiatry*, 36 Suppl 3, S207-S214.
- Swaab, D. F., Bao, A. M., and Lucassen, P. J. (2005). The stress system in the human brain in depression and neurodegeneration. *Ageing Res.Rev.*, 4, 141-194.
- Van Der Wee, N. J., Fiselier, J., van Megen, H. J., and Westenberg, H. G. (2004). Behavioural effects of rapid intravenous administration of meta-chlorophenylpiperazine in patients with panic disorder and controls. *Eur. Neuropsychopharmacol.*, 14, 413-417.
- Vaswani, M., Linda, F. K., and Ramesh, S. (2003). Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. *Prog. Neuropsychopharmacol. Biol.Psychiatry*, 27, 85-102.
- Wittchen, H. U. and Fehm, L. (2003). Epidemiology and natural course of social fears and social phobia. *Acta Psychiatr.Scand.Suppl*, 4-18.
- Wittchen, H. U., Stein, M. B., and Kessler, R. C. (1999). Social fears and social phobia in a community sample of adolescents and young adults: prevalence, risk factors and co-morbidity. *Psychol.Med.*, 29, 309-323.

**Behavioural effects of rapid
intravenous administration of meta-
chlorophenylpiperazine (m-CPP) in patients
with generalized social anxiety disorder,
panic disorder and healthy controls**

European Neuropsychopharmacology 2007;17:637-642

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Abstract

Findings from epidemiological, pharmacotherapeutical, genetic and neurobiological studies suggest a possible overlap in the neurobiology of generalized social anxiety disorder (gSAD) and panic disorder (PD). Previously we have found a rapid intravenous m-CPP challenge of 0.1 mg/kg to be highly sensitive and selective in the provocation of panic attacks in patients with PD. We therefore directly compared the behavioural, neuroendocrine and physiological effects of this rapid m-CPP challenge in a small sample of patients with gSAD, patients with PD and matched healthy controls. Panic attacks were significantly more provoked in patients with PD (85%), but not in patients with gSAD (14%) as compared 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. Our preliminary data do not support a shared neurobiology of gSAD and PD.

Introduction

Generalized social anxiety disorder (gSAD) and panic disorder (PD) are among the most prevalent anxiety disorders, with reported lifetime prevalences in Europe of 2.4% for SAD and of 2.1% for PD (Alonso et al., 2004a). In the United States lifetime prevalences of 3.4% for PD and 13.3% for SAD were found (Sheikh et al., 2002; Magee et al., 1996). PD and gSAD may cause severe social, occupational and academic impairment and typically have a chronic course. Although the two disorders clearly have a different core phenomenology, with spontaneous panic attacks occurring in PD and fear of scrutiny by others in gSAD, data from epidemiological, pharmacotherapeutical, genetic as well as a variety of neurobiological studies suggest an overlap in the neurobiology of gSAD and PD.

In epidemiological studies gSAD and PD are usually found to be highly comorbid conditions. Thus, in the European Study of the epidemiology of mental disorders (ESEMEd) the 12-month pair wise association between SAD and PD expressed in odd ratio's was 11.6 (Alonso et al., 2004b). Comparable to these findings in adult populations, the results of a recent large study in pre-adolescents indicated that, in a general population sample, it may not be useful to discern children with different types of anxiety symptoms (Ferdinand et al., 2006).

Pharmacotherapeutical studies have shown the efficacy of the selective serotonin reuptake inhibitors (SSRIs) in gSAD and PD, implicating the involvement of the serotonergic system in both disorders. However, tricyclic antidepressants and alprazolam have been found to be less effective in gSAD than in PD (Blanco et al., 2003; Kasper and Resinger, 2001; Zohar and Westenberg, 2000). At large, genetic studies seem to point at an anxiety diathesis model, i.e. a genetic predisposition to develop anxiety related symptoms and anxiety disorders. There seem to be genes that increase the risk only for specific disorders, as well as genes that increase the risk for anxiety disorders in general (Villafructe and Burmeister, 2003; Hettema et al., 2005). Neuroimaging studies have shown the involvement of the same fear-circuitry in PD and in gSAD, but some differences have been found, notably in the involvement of elements of the dopaminergic system (Kent and Rauch, 2003; Charney, 2003).

A large number of studies on the neurobiology of gSAD and PD has employed challenge paradigms with anxiogenic or panicogenic pharmacological agents, often resulting in more or less comparable behavioural effects in patients with PD and patients with gSAD. However, only a small number of these studies directly compared the effects of the panicogenic challenge in patients with PD, patients with gSAD and matched healthy controls (Gorman et al., 1990; Papp et al., 1993; Caldirola et al., 1997; McCann et al., 1997; Tancer et al., 1994). We studied the effects of 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 (α_2) adrenergic and dopamine receptors. We found this rapid intravenous m-CPP challenge to be highly sensitive and selective in the provocation of panic attacks in patients with PD as compared to healthy controls (panic attacks were provoked in 90% of the controls and in 0 % of the healthy controls) (Van Der Wee et al., 2004). We therefore decided to further elucidate the putative shared neurobiology of

gSAD and PD by directly comparing the behavioural, neuroendocrine and physiological effects of the rapid intravenous administration of 0.1 mg/kg m-CPP in patients with gSAD, patients with PD, and matched healthy controls.

Experimental procedures

Subjects

Seven patients (five males, two females) with gSAD, seven patients with PD with or without agoraphobia and seven healthy controls participated in this study. Subjects were pair wise matched for sex, and group-wise on age. The diagnosis was made according to DSM-IV criteria, no axis I and no major axis II comorbidity was allowed and the diagnosis was confirmed by the Mini International Neuropsychiatric Interview Plus 5.0.0 (Sheehan et al., 1998; Van Vliet and De Beurs, 2007). In addition, no life time comorbidity between PD and gSAD was allowed. Subjects had no clinically significant medical disorders, were drug free for minimal 2 weeks (60 days for fluoxetine, six months for corticosteroids), had not donated blood during the 60 days preceding the test day, female subjects were not pregnant or breast-feeding, and all subjects had normal physical and laboratory examinations. There were no subjects with a history or suspicion of substance abuse. Subjects using drugs of abuse or more than 6 cups of coffee, 15 cigarettes or 3 units of alcohol a day, were excluded. The study was performed in the outpatient clinic of the University Medical Center Utrecht, the Netherlands, and was approved by the Medical Ethical Committee of the University Medical Center Utrecht. All subjects gave written informed consent prior to inclusion in the study.

Procedures

We used the same single blind, comparative design, as in our previous study (Van Der Wee et al., 2004). Subjects were told that they would receive either m-CPP or a solution mimicking some of the side-effects of m-CPP (i.e. hot and cold flushes and dizziness). In reality all subjects received m-CPP. Subjects took a light breakfast at least one hour before the test. Coffee, smoking and alcoholic beverages were not allowed from 9 p.m. on the evening before. Immediately after baseline assessments an indwelling intravenous catheter was placed in a forearm vein in each arm at 9.00 a.m. At 10.00 a.m. m-CPP (0.1 mg/kg diluted in 20 ml of normal saline) was administered in 90 seconds by means of an automatic pump (Becton Dickinson). Behavioural, physiological and neuroendocrine responses, as well as m-CPP plasma levels were measured immediately before infusion and at 30-minutes intervals until 150 minutes after infusion.

Behavioural assessments

Behavioural responses were measured prior to the measurement of physiological and neuroendocrine responses. Behavioural responses were assessed by using a Visual Analogue Scale (VAS) for anxiety and the Panic Symptom Scale (PSS) (Bradwejn et al., 1992; Van Megen et al., 1994; Van Megen et al., 1996). The VAS for anxiety was used to evaluate the change in anxiety, with a score range from 0 =

not at all to 100 = most ever. The PSS is a self-rating instrument derived from DSM-III-R criteria for a panic attack. Both the symptom severity and the fear of the symptom are rated on a 5-point scale (0 = not at all to 4 = severe).

After the challenge the occurrence of panic attacks (the main outcome measure) was assessed. A panic attack had to fulfil the following criteria: subjects had to experience a feeling of panic, had to have an increase of at least four of the 13 DSM-IV symptoms of a panic attack, as extracted from the PSS, combined with a score of two or more on the item 'Apprehension' of these four symptoms. Subjects had to report that the panic attack was similar to their spontaneous ones when applicable.

Vital signs

Temperature (orally measured), systolic and diastolic blood pressure (supine after 5 minutes rest; standing after 1 minute standing), and heart rate (supine after 5 minutes rest; standing after 1 minute standing) were recorded. Blood pressure and heart rate measurements were assessed with a completely automated device consisting of an inflatable cuff and an oscillatory detection system. All values were registered on a built-in recorder so that measurements are observer-independent.

Neuroendocrine parameters

Neuroendocrine measurements consisted of assessment of cortisol and growth hormone (GH) levels. Cortisol was measured using a competitive, chemiluminescent assay (ACS: Centaur Cortisol, Chiron Diagnostics Corporation, East Walpole, MA, USA). The intra-assay and inter-assay coefficients of variation at 4 µg/ml were 4% and 6% respectively. GH was assayed using a commercially available radio-immunoassaykit (Oris Industry Company, Gif-sur-Yveth, France), with a lower limit of detection of 0.5 mU/l and an intra- and interarray coefficient of variation of 8 and 11% respectively.

Pharmacokinetics

M-CPP was kindly provided by Janssen Pharmaceuticals. M-CPP plasma levels were taken 30, 60, 90, 120 and 150 minutes after m-CPP administration, and analysed using a high-performance liquid chromatography procedure as described by Suckow et al. (1990) and slightly modified to the use of an electrochemical detector.

Statistics

Since the data were not normally distributed, non-parametric statistics were used. The rate of panic attacks in the three groups following the administration of m-CPP was compared using a Fisher's exact test for three groups, followed by Fisher's exact tests for two groups when a significant result was obtained. P-values were Bonferroni corrected for multiple comparisons.

Delta scores (Δ , defined as the maximum change from baseline) were calculated for the PSS, for the VAS anxiety and for cortisol and GH. Delta scores and age and peak levels of m-CPP for the three groups were first analysed with a Kruskal-Wallis test, followed by Mann-Whitney U tests when a significant result was obtained. A non-significant result from the Kruskal-Wallis tests for a delta score could indicate a similar change across the three groups over time as well as the absence

of any effect of the m-CPP administration. Therefore, in the case of a non-significant result from the Kruskal-Wallis test, a post-hoc Friedman test was performed to assess that there had been an effect of m-CPP on this specific parameter over time.

Results

The three groups did not significantly differ in age ($p=0.786$; gSAD mean age $40.9 \text{ years} \pm 14.6$; PD 39.0 ± 8.8 ; Co 37.1 ± 7.8). We found no differences in peak m-CPP levels ($p=0.573$; gSAD mean $43.0 \text{ ng/ml} \pm 24.9$; PD 44.2 ± 11.9 ; Co 36.4 ± 10.4). Six out of seven PD patients (85 %), one out of seven gSAD patients (14%) and none of the healthy controls experienced a panic attack following the rapid intravenous administration of m-CPP. This was a highly significant between group difference (Fisher's exact test, $p=0.003$). Fisher's exact tests on two groups showed a significant difference in panic attack rate between gSAD and PD ($p=0.045$, Bonferroni corrected) and between PD and controls ($p=0.006$, Bonferroni corrected), but not between gSAD and controls ($p=1.5$, Bonferroni corrected).

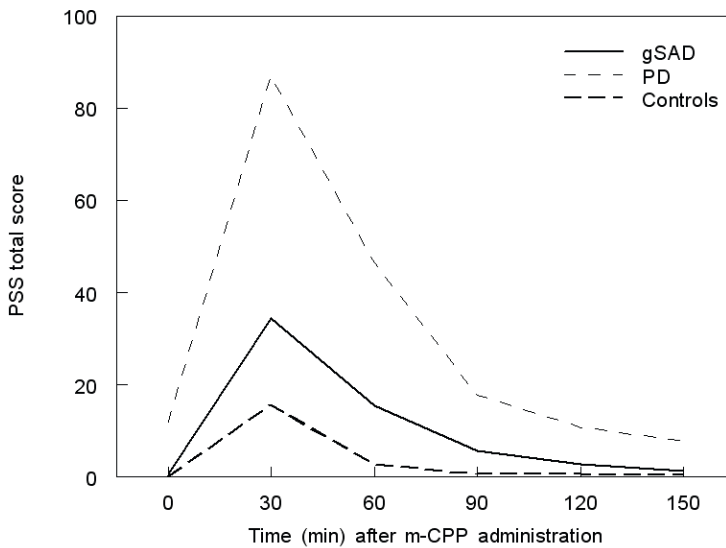


Figure 1 PSS total scores after m-CPP administration. gSAD is generalized social anxiety disorder, PD is panic disorder and Co is controls. M-CPP is administered at $t=0$.

Analysis of the Δ PSS total score and the Δ VAS anxiety with a Kruskal-Wallis test yielded a significant difference for the Δ PSS total score ($p=0.001$) and for the Δ VAS anxiety ($p=0.047$). Post-hoc analysis with Mann-Whitney U tests showed significant differences on the Δ PSS total score between gSAD and PD ($p=0.017$), between gSAD and controls ($p=0.038$), and between PD and controls ($p=0.001$). The PD group showed the highest Δ PSS score and the control group the lowest. For details see Figure 1, and Tables 1 and 2.

Post-hoc analysis on the Δ VAS anxiety showed significant differences between PD and controls ($p=0.038$) and almost reached significance between gSAD and controls ($p=0.053$). No significant difference was found on the Δ VAS anxiety between PD and gSAD ($p=0.383$). For details see Tables 1 and 2. In one patient with gSAD who experienced no panic attack, blood could not be obtained at all time points. This patient was not included in the analysis of the neuro-endocrine parameters and the closest match for sex and age in the other groups was removed by a researcher blinded for the results of the assessments (I.V.). The Kruskal-Wallis test showed a significant difference for Δ growth hormone ($p=0.042$), but not for Δ cortisol ($p=0.371$). Δ GH responses were significant different between gSAD and PD ($p=0.015$), but not between both patient groups and controls. Of the three groups the gSAD group had the highest Δ GH levels after m-CPP administration and the PD group the lowest, with the controls in between. For details see tables 1 and 2.

Post-hoc Friedman test for cortisol levels showed a significant effect of m-CPP administration on cortisol levels ($p=0.000$). For details see Tables 1 and 2.

We found no differences between the three groups in changes in the physiological parameters.

Table 1 Mean Δ scores and standard deviation after m-CPP administration of PSS total score, VAS anxiety, growth hormone and cortisol

	gSAD	PD	Co	p (KW)
Δ PSS total score	33.9 ± 20.9	75.1 ± 31.2	15.4 ± 8.1	0.001*
Δ VAS anxiety	31.6 ± 25.4	46.1 ± 32.1	12.7 ± 25.9	0.047*
Δ GH (mU/l)	23.1 ± 20.0	8.8 ± 9.0	7.6 ± 9.4	0.042*
Δ Cortisol ($\mu\text{g/ml}$)	0.15 ± 0.12	0.23 ± 0.14	0.26 ± 0.14	0.371

gSAD is social anxiety disorder, PD is panic disorder and Co is controls. PSS is the Panic Symptom Scale rating the presence of a symptom and the fear provoked by a symptom. VAS is the Visual Analogue Scale for anxiety. GH is growth hormone. For all comparisons a Kruskal-Wallis (KW) test was used. Δ is the difference between the baseline value and the maximum value after m-CPP administration. Mean Δ scores and standard deviation are given instead of medians or mean ranks, in order to give better insight in the data. P-values are presented uncorrected for multiple comparisons.

Table 2 P-values of post-hoc Mann-Whitney U tests of Δ PSS total scores, Δ VAS anxiety, and Δ GH.

	p gSAD : PD	p gSAD : Co	p PD : Co
Δ PSS total score	0.017*	0.038*	0.001*
Δ VAS anxiety	0.383	0.053	0.038*
Δ GH	0.015*	0.138	0.805

gSAD is generalized social anxiety disorder, PD is panic disorder and Co is controls. PSS is the Panic Symptom Scale rating the presence of a symptom and the fear provoked by a symptom. VAS is the Visual Analogue Scale for anxiety. GH is growth hormone. For post-hoc 2x2 comparisons a two-tailed Mann-Whitney U test was used. P-values are presented uncorrected for multiple comparisons.

Discussion

Using a rapid i.v. m-CPP challenge we found a different behavioural and neurobiological response in gSAD and PD. The challenge resulted in a high frequency of panic attacks and high PSS scores in the PD group, while the gSAD group experienced panic attacks in a very low frequency, comparable to the controls.

Our results differ from findings in previous studies comparing the effects of panicogenic challenges in gSAD and PD. Several anxiogenic challenges, like 35% CO₂, pentagastrin and caffeine, did not show significant differences in the occurrence of panic attacks between gSAD and PD (Caldirola et al., 1997; Gorman et al., 1990; McCann et al., 1997; Tancer et al., 1994). In other panicogenic challenges with 5% CO₂, 35% CO₂, lactate infusions and hyperventilation, the gSAD group showed less panic attacks than the PD group, but more than the control group when a control group was available (Holt and Andrews, 1989; Liebowitz et al., 1985; Papp et al., 1993). Some studies also employed scales to measure the provoked levels of anxiety. In these studies, with pentagastrin, caffeine and 35 % CO₂, no differences in anxiety ratings were found between gSAD and PD (Caldirola et al., 1997; Gorman et al., 1990; Tancer et al., 1994). Only in a challenge study with 35 % CO₂, a pattern similar to the one found in our study was found, with anxiety levels being the highest in PD, intermediate in gSAD and the lowest in the control group, although this effect reached significance only when sex was not part of the analysis (Papp et al., 1993).

Neuroendocrine measures were only included in a few studies examining pharmacological panicogenic challenges in gSAD, with unequivocal results. Thus, following a pentagastrin challenge, no differences among groups (gSAD, PD and controls) in cortisol responses were found (McCann et al., 1997). However, after an (orally administered) m-CPP challenge, female patients with gSAD showed more robust cortisol responses than female controls (Hollander et al., 1998). After the administration of caffeine, differences were found between the cortisol and lactate responses in patients with gSAD, patients with PD and healthy controls. The cortisol response was the highest in PD and the lowest in controls, with the response in gSAD being intermediate. The lactate response was also the highest in PD patients, but lowest in gSAD, with the controls in between (Tancer et al., 1994).

A challenge with clonidine resulted in blunted GH responses in gSAD and PD as compared to controls (Tancer et al., 1993). In the present study we found an augmented GH response to m-CPP in gSAD compared to PD, with the control group in between. However, GH levels may be difficult to interpret because of the pulsatile secretion and the possible confound of the occurrence of nausea, a common effect of m-CPP.

The present study suffers from some limitations. We did not use a placebo-controlled design. However, to minimize the occurrence of spontaneous panic attacks, subjects were given the impression that they might also receive a saline solution mimicking the initial effects of m-CPP. Moreover, in previous placebo-controlled challenge studies in patients with gSAD and PD at our center, using the same type of experimental procedure, a placebo response of 0 % was found (Van Megen et al., 1994; Van Vliet et al., 1997). All other placebo-controlled m-CPP challenge studies

in patients with PD also found placebo responses of 0 % (Charney et al., 1987; Germaine et al., 1994; Kahn et al., 1988; Klein et al., 1991; Wetzler et al., 1996). These findings suggest that the panicogenic effect of the experimental procedure itself is very small.

Behavioural assessments were made at 30 minutes intervals and for the first 30 minutes interval following the i.v. m-CPP administration retrospectively. In view of the time of onset and duration of symptoms a shorter interval, i.e. ten minutes, would have been more appropriate for behavioural assessments during the first hour after i.v. m-CPP administration.

Our neuroendocrine assessments consisted only of cortisol and growth hormone. We did not assess prolactin, which might be a more reliable index of 5-HT stimulation. In the present study the differences in cortisol responses to the m-CPP challenge failed to reach statistical significance, probably as a result of a ceiling effect occurring with higher plasma levels of m-CPP.

Like several other challenge studies in PD, our study had a drug free period of at least two weeks. Several patients were off medication for a longer period of time. As a drug free period of two weeks may be too short to allow for a complete readaptation of the receptors after long-term antidepressant treatment, this may be a potential confounding factor

Finally, our design did not allow for a separation between biochemically and cognitively mediated effects of our rapid i.v. m-CPP administration. As i.v. m-CPP is known to cause symptoms like light-headedness, nausea and hot and cold flushes in healthy controls, part of its effects in panic disorder might be attributable to cognitive factors like the misinterpretation of bodily symptoms (Austin and Richards, 2001). However, after the rapid i.v. administration of m-CPP most somatic symptoms were present to a minimal extent in controls and in the gSAD group. Furthermore, several somatic symptoms were only reported by the patients with PD.

Although preliminary, our data support a distinction between gSAD and PD on a neurobiological level and confirm that panic attacks following the rapid i.v. 0.1 mg/kg m-CPP challenge test combine high sensitivity and selectivity for PD. Future research should replicate our findings in a larger sample size and in a placebo-controlled, double-blind design. It will also be important to conduct comparative studies of PD versus gSAD with a subtyping of gSAD patients in panic or non panic type, to evaluate whether a history of panic attacks or the diagnostic category explains the difference in the rate of m-CPP provoked panic attacks.

References

- Alonso, J., Angermeyer, M. C., Bernert, S., Bruffaerts, R., Brugha, T. S., Bryson, H. et al. (2004a). Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. *Acta Psychiatr.Scand.Suppl*, 21-27.
- Alonso, J., Angermeyer, M. C., Bernert, S., Bruffaerts, R., Brugha, T. S., Bryson, H. et al. (2004b). 12-Month comorbidity patterns and associated factors in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. *Acta Psychiatr.Scand.Suppl*, 28-37.
- Austin, D. W. and Richards, J. C. (2001). The catastrophic misinterpretation model of panic disorder. *Behav.Res. Ther.*, 39, 1277-1291.
- Blanco, C., Raza, M. S., Schneier, F. R., and Liebowitz, M. R. (2003). The evidence-based pharmacological treatment of social anxiety disorder. *Int.J.Neuropsychopharmacol.*, 6, 427-442.
- Bradwejn, J., Koszycki, D., Annable, L., Couetoux du, T. A., Reines, S., and Karkanas, C. (1992). A dose-ranging study of the behavioral and cardiovascular effects of CCK-tetrapeptide in panic disorder. *Biol.Psychiatry*, 32, 903-912.
- Caldirola, D., Perna, G., Arancio, C., Bertani, A., and Bellodi, L. (1997). The 35% CO₂ challenge test in patients with social phobia. *Psychiatry Res.*, 71, 41-48.
- Charney, D. S. (2003). Neuroanatomical circuits modulating fear and anxiety behaviors. *Acta Psychiatr.Scand.Suppl*, 38-50.
- Charney, D. S., Woods, S. W., Goodman, W. K., and Heninger, G. R. (1987). Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology (Berl)*, 92, 14-24.
- Ferdinand, R. F., van Lang, N. D., Ormel, J., and Verhulst, F. C. (2006). No distinctions between different types of anxiety symptoms in pre-adolescents from the general population. *J.Anxiety.Disord.*, 20, 207-221.
- Germine, M., Goddard, A. W., Sholomskas, D. E., Woods, S. W., Charney, D. S., and Heninger, G. R. (1994). Response to meta-chlorophenylpiperazine in panic disorder patients and healthy subjects: influence of reduction in intravenous dosage. *Psychiatry Res.*, 54, 115-133.
- Gorman, J. M., Papp, L. A., Martinez, J., Goetz, R. R., Hollander, E., Liebowitz, M. R. et al. (1990). High-dose carbon dioxide challenge test in anxiety disorder patients. *Biol.Psychiatry*, 28, 743-757.
- Hettema, J. M., Prescott, C. A., Myers, J. M., Neale, M. C., and Kendler, K. S. (2005). The structure of genetic and environmental risk factors for anxiety disorders in men and women. *Arch.Gen.Psychiatry*, 62, 182-189.
- Hollander, E., Kwon, J., Weiller, F., Cohen, L., Stein, D. J., DeCaria, C. et al. (1998). Serotonergic function in social phobia: comparison to normal control and obsessive-compulsive disorder subjects. *Psychiatry Res.*, 79, 213-217.
- Holt, P. E. and Andrews, G. (1989). Provocation of panic: three elements of the panic reaction in four anxiety disorders. *Behav.Res.Ther.*, 27, 253-261.
- Kahn, R. S., Asnis, G. M., Wetzler, S., and van Praag, H. M. (1988). Neuroendocrine evidence for serotonin receptor hypersensitivity in panic disorder. *Psychopharmacology (Berl)*, 96, 360-364.
- Kasper, S. and Resinger, E. (2001). Panic disorder: the place of benzodiazepines and selective serotonin reuptake inhibitors. *Eur.Neuropsychopharmacol.*, 11, 307-321.
- Kent, J. M. and Rauch, S. L. (2003). Neurocircuitry of anxiety disorders. *Curr.Psychiatry Rep.*, 5, 266-273.

- Klein, E., Zohar, J., Geraci, M. F., Murphy, D. L., and Uhde, T. W. (1991). Anxiogenic effects of m-CPP in patients with panic disorder: comparison to caffeine's anxiogenic effects. *Biol Psychiatry*, 30, 973-984.
- Liebowitz, M. R., Fyer, A. J., Gorman, J. M., Dillon, D., Davies, S., Stein, J. M. et al. (1985). Specificity of lactate infusions in social phobia versus panic disorders. *Am.J.Psychiatry*, 142, 947-950.
- Magee, W. J., Eaton, W. W., Wittchen, H. U., McGonagle, K. A., and Kessler, R. C. (1996). Agoraphobia, simple phobia, and social phobia in the National Comorbidity Survey. *Arch.Gen.Psychiatry*, 53, 159-168.
- McCann, U. D., Slate, S. O., Geraci, M., Roscow-Terrill, D., and Uhde, T. W. (1997). A comparison of the effects of intravenous pentagastrin on patients with social phobia, panic disorder and healthy controls. *Neuropsychopharmacology*, 16, 229-237.
- Papp, L. A., Klein, D. F., Martinez, J., Schneier, F., Cole, R., Liebowitz, M. R. et al. (1993). Diagnostic and substance specificity of carbon-dioxide-induced panic. *Am.J.Psychiatry*, 150, 250-257.
- Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E. et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J.Clin.Psychiatry*, 59 Suppl,%20:22-33;quiz 34-57., 22-33.
- Sheikh, J. I., Leskin, G. A., and Klein, D. F. (2002). Gender differences in panic disorder: findings from the National Comorbidity Survey. *Am.J.Psychiatry*, 159, 55-58.
- Suckow, R.F., Cooper, T.B. and Kahn, R.S. (1990). High-performance liquid chromatographic method for the analysis of plasma m-chlorophenylpiperazine. *J.Chromatogr.* 528, 228-234.
- Tancer, M. E., Stein, M. B., and Uhde, T. W. (1993). Growth hormone response to intravenous clonidine in social phobia: comparison to patients with panic disorder and healthy volunteers. *Biol.Psychiatry*, 34, 591-595.
- Tancer, M. E., Stein, M. B., and Uhde, T. W. (1994). Lactic acid response to caffeine in panic disorder: comparison with social phobics and normal controls. *Anxiety*, 1, 138-140.
- Van Der Wee, N. J., Fiselier, J., van Megen, H. J., and Westenberg, H. G. (2004). Behavioural effects of rapid intravenous administration of meta-chlorophenylpiperazine in patients with panic disorder and controls. *Eur. Neuropsychopharmacol.*, 14, 413-417.
- Van Megen, H. J., Westenberg, H. G., and den Boer, J. A. (1996). Effect of the cholecystokinin-B receptor antagonist L-365,260 on lactate-induced panic attacks in panic disorder patients. *Biol.Psychiatry*, 40, 804-806.
- Van Megen, H. J., Westenberg, H. G., den Boer, J. A., Haigh, J. R., and Traub, M. (1994). Pentagastrin induced panic attacks: enhanced sensitivity in panic disorder patients. *Psychopharmacology (Berl)*, 114, 449-455.
- Van Vliet, I. M. and De Beurs, E. (2007). [The MINI-International Neuropsychiatric Interview. A brief structured diagnostic psychiatric interview for DSM-IV en ICD-10 psychiatric disorders]. *Tijdschr.Psychiatr.*, 49, 393-397.
- Van Vliet, I. M., Westenberg, H. G., Slaap, B. R., den Boer, J. A., and Ho Pian, K. L. (1997). Anxiogenic effects of pentagastrin in patients with social phobia and healthy controls. *Biol.Psychiatry*, 42, 76-78.
- Villafuerte, S. and Burmeister, M. (2003). Untangling genetic networks of panic, phobia, fear and anxiety. *Genome Biol.*, 4, 224.
- Wetzler, S., Asnis, G. M., DeLecuona, J. M., and Kalus, O. (1996). Serotonin function in panic disorder: intravenous administration of meta-chlorophenylpiperazine. *Psychiatry Res.*, 64, 77-82.
- Zohar, J. and Westenberg, H. G. (2000). Anxiety disorders: a review of tricyclic antidepressants and selective serotonin reuptake inhibitors. *Acta Psychiatr.Scand.Suppl*, 403:39-49., 39-49.

**Increased serotonin and dopamine
transporter binding in psychotropic
medication-naïve patients with generalized
social anxiety disorder shown by ^{123}I - β -(4-
iodophenyl)-tropane SPECT**

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Abstract

There is circumstantial evidence for the involvement of serotonergic and dopaminergic systems in the pathophysiology of social anxiety disorder. In the present study, using SPECT imaging, we examined the ^{123}I - β -(4-iodophenyl)-tropane binding potential for the serotonin and dopamine transporter in patients with a generalized social anxiety disorder and age- and sex- matched healthy controls. Methods: Twelve psychotropic medication-naïve patients with social anxiety disorder, generalized type (5 women and 7 men) and 12 sex- and age- matched healthy controls were studied. Volumes of interest were constructed on MRI-coregistered SPECT scans. Binding ratios were compared using the Mann-Whitney U test. Possible correlations between binding patterns and symptomatology were assessed using the Spearman rank correlation coefficient. Results: Significantly higher binding potentials were found for the serotonin transporter in the left and right thalamus of patients. Patients had also a significantly higher binding potential for the dopamine transporter in the striatum. Conclusion: The present study provided direct evidence for abnormalities in both the dopaminergic and the serotonergic systems in patients with generalized social anxiety disorder.

Introduction

Social anxiety disorder (also known as social phobia) is a disabling condition that afflicts a large part of the general population. It tends to run a chronic and unremitting course and often leads to the development of alcoholism and depression. The essential feature of social anxiety disorder is the fear of being evaluated by others with the expectation that such an assessment will be negative and embarrassing. Social anxiety disorder has been subdivided into two subtypes. The first subtype, referred to in the DSM-IV (American Psychiatric Association, 1994) as 'generalized' social phobia, involves fear of a broad array of social situations. The second subtype, referred to as discrete or specific social anxiety disorder, is usually confined to one or two performance situations, of which public speaking is the most common (Westenberg, 1998). Given the clinical importance of social anxiety disorder, the neurobiology of this condition has received little attention to date.

Treatment studies demonstrating that selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors are effective in social anxiety disorder hint that serotonergic and catecholaminergic pathways have a role, but these findings can be only a rough guide in determining the neurobiology. Challenge tests with fenfluramine and m-chlorophenylpiperazine have provided other circumstantial evidence for the role of serotonin (5-hydroxytryptamine or 5-HT) in social anxiety disorder (Tancer et al., 1994; Hollander et al., 1998). An involvement of the dopaminergic system in social anxiety was suggested by findings that homovanillic acid levels in cerebrospinal fluid tended to be lower in panic disorder patients with comorbid social anxiety disorder than in those without (Johnson et al., 1994). Moreover, the prevalence of social anxiety disorder is increased in patients in whom Parkinson's disease develops (Stein et al., 1990). More recently, two neuroimaging studies have provided direct evidence that dopamine systems may play a role in the neurobiology of social anxiety disorder. Using ^{123}I -labeled 2- β -carbomethoxy-3- β -(4-iodophenyl)-tropane (^{123}I - β -CIT) as a tracer and SPECT, Tiihonen et al. found that the density of the dopamine transporter (DAT) in the striatum was reduced in patients with generalized social anxiety disorder (Tiihonen et al., 1997). Schneier et al., using ^{123}I -iodobenzamide SPECT, found a reduced dopamine D_2 binding potential in this psychiatric condition (Schneier et al., 2000). Although neuroimaging studies potentially could also provide direct evidence for a role of serotonergic systems in social anxiety disorder, to our knowledge only one such study has been published to date (Lanzenberger et al., 2007). In this study, by Lanzenberger et al., 5-HT receptor 1A binding in several limbic and paralimbic areas was found to be reduced in social anxiety disorder.

^{123}I - β -CIT SPECT can be used to visualize both DAT and 5-HT transporter (5-HTT) in the human brain after a single administration of the ligand. Binding of ^{123}I - β -CIT in the striatal region has been shown to reflect mainly binding to DAT; binding in the thalamus, midbrain, and pons reflects predominantly binding to 5-HTT (Pirker et al., 1995; De Win et al., 2005). The binding to DAT and 5-HTT can be further differentiated by using the difference in time course of ^{123}I - β -CIT uptake in DAT- and 5-HTT-rich brain regions (Pirker et al., 1995). In the present

study, we used this approach to investigate DAT and 5-HTT binding potentials in right-handed psychotropic medication-naïve patients with generalized social anxiety disorder (according to DSM-IV criteria) and no comorbidity and in healthy controls matched pair wise by age, sex, and handedness. We expected the binding pattern of ^{123}I - β -CIT to reflect abnormalities at the level of both 5-HTT and DAT.

Materials and Methods

Subjects

The study was approved by the ethics committee of the University Medical Center, Utrecht, The Netherlands, and was performed in accordance with the ethical standards of the declaration of Helsinki. After a complete description of the study had been provided to the subjects, written informed consent was obtained. The patients came from direct physician referrals to our specialized anxiety clinic or reacted to advertisements. Healthy controls were enrolled through advertisements in flyers and newspapers or obtained from an existing database. Only subjects without a lifetime history of psychosis, substance abuse, recurrent major depression, bipolar disorder, eating disorders, other anxiety disorders, tics, and stuttering were included. All participants had no lifetime history of illnesses with possible central nervous system sequelae and were in good physical health, as confirmed by physical and laboratory examinations. Subjects consumed fewer than 6 cups of coffee and 3 units of alcohol a day and smoked fewer than 6 cigarettes a day. Screening for current and prior adult psychopathology was done by administering the Mini International Neuropsychiatric Interview Plus, version 5.0.0 (Sheehan et al., 1998). Diagnoses were confirmed by an experienced clinician. In addition, the Liebowitz Social Anxiety Scale (LSAS) was used to assess the severity of the social anxiety symptoms at entry (Heimberg et al., 1999). Handedness was determined by administering the Edinburgh Handedness Scale (Oldfield, 1971).

Subjects were excluded when they had a score of more than 13 on the 17-item Hamilton Depression Rating Scale (Hamilton, 1967). Subjects underwent imaging within 2 weeks after inclusion. Any cognitive behavioral therapy had been terminated at least 3 months before the study.

Twelve patients and 12 controls were enrolled. All subjects completed the study. The patients and controls were perfectly matched for sex and did not differ significantly in age and handedness. Demographic and clinical characteristics are shown in Table 1.

Table 1 Demographic and Clinical Characteristics of Study Population.

Characteristic	Patients (n=12)	Controls (n=12)
Mean age \pm SD (y)	39.4 \pm 12.6	33.0 \pm 9.5
Total Edinburgh Handedness Scale	0.95 \pm 0.04	0.96 \pm 0.03
Men (n)	7	7
Women (n)	5	5
Nonsmokers (n)	9	8
Length of illness (y)	23.9 \pm 14.7	
Total LSAS score	73.6 \pm 13.7	
Total Hamilton Depression Rating Scale	8.3 \pm 2.1	

Image acquisition and analysis

Images were acquired and analyzed using the same methods as in our previously reported study on patients with obsessive-compulsive disorder (Van der Wee et al., 2004). On the first day of scanning, the subjects received an intravenous injection of approximately 150 MBq of ^{123}I - β -CIT (MAP Medical Technologies; radionuclidic purity [^{125}I / ^{123}I] of at least 9.5×10^{-3} at calibration time and a radiochemical purity of at least 95 %). We used a Prism 3000 triple-head γ -camera (Picker) with ultra-high-resolution fanbeam collimators and a full width at half maximum of approximately 12 mm. Four hours after the injection, the first scan was made to assess binding to 5-HTT. Between 22 and 24 hours after the injection, the second scan was obtained to measure binding to DAT (Kuikka et al., 1995; Laruelle et al., 1994; Willeit et al., 2000). The subjects refrained from coffee and nicotine in the 6-10 hours preceding each SPECT scan. Immediately after the first scan, the subjects received 20 mg of paroxetine to displace the ^{123}I - β -CIT from 5-HTT, so that binding to DAT could be determined more precisely (Kuikka et al., 1995). Several studies have demonstrated that at modest dosages (e.g. 10 mg) of paroxetine and other potent 5-HT reuptake blockers, occupation of 5-HTT is already virtually maximal (Pirker et al., 1995; Tauscher et al., 2001; Meyer et al., 2001). To control for possible differences in metabolism between subjects, we chose a higher oral dose of 20 mg. The 20-mg dose of paroxetine was well tolerated by all subjects. During scanning, subjects were supine with eyes and ears open and head fixed in a head holder. We ensured that the patients stayed awake and did not move. For an accurate determination of each subject's volumes of interest (VOIs), all subjects also underwent structural MRI (3-dimensional fast field echo; echo time/ repetition time, 4.6/30 ms; flip angle, 30°; field of view, 256 x 256 mm; matrix, 128 x 128 x 130 mm; slice thickness, 2 mm) 2 hours before the injection of ^{123}I - β -CIT. The MRI scans were reoriented to the standardized coordinate system of the Montreal standard brain (Collins et al., 1994). VOIs were delineated manually on the reoriented MRI scans by a researcher who was unaware of the subject's identity and diagnosis, by means of the display software from the Brain Imaging Center of the Montreal Neuroimaging Institute (MacDonald, 1996). Because

the focus of our study was putative abnormalities at the level of 5-HTT in Social Anxiety Disorder, the VOIs for 5-HTT included the left and right thalamus and the midbrain/pons region, whereas we limited the VOI for DAT to the left and right striatum taken together. Furthermore, this choice allowed a direct comparison of DAT findings with the previous study of Tiihonen et al. (Tiihonen et al., 1997). We planned an exploratory posthoc analysis in which left and right striatal subregions would be delineated in cases in which the ROI striatum showed a significant difference between patients and controls. The cerebellum was used as a reference region, representing the nonspecific binding for ^{123}I - β -CIT.

To allow exact coregistration of MRI and SPECT scans, we used fiducial markers. Fiducial markers were cone-shaped, with cross-shaped feet, and were placed on the nose bridge and preauricular above the mandibular joints. The position of each marker was indicated with four dots on subject's skin to allow for repositioning of markers immediately before the SPECT scans. Vitamin A and ^{57}Co were used as contrast agents for the MRI and SPECT scans, respectively. The energy was set at a peak of 160 keV with a window of 20% for ^{123}I - β -CIT and at a peak of 120 keV with a window of 15% for ^{57}Co . After standard processing, brain SPECT images were resliced to isotropic voxels with dimensions of 2 mm and further treated as 3D volumes to coregister within the 3-dimensional orientation of the MRI scans. Coregistration was performed semiautomatically and was based on the position of the fiducial markers, using the Register multimodality software package and additional software developed at the Brain Imaging Center of the Montreal Neuroimaging Institute (MacDonald, 1993). The researcher performing the coregistration was unaware of subject identity and diagnosis.

For each separate VOI, the ratio of specific binding of ^{123}I - β -CIT to 5-HTT or DAT was calculated according to methodology used in previously published ^{123}I - β -CIT studies: the average radioactivity count per voxel per VOI minus the average radioactivity count per voxel in the cerebellum, divided by the average radioactivity count per voxel in the cerebellum.

Statistical analysis

Age was compared using the Student *t* test. The interrater and intrarater reliability for VOI registration was assessed by calculating the intraclass correlation coefficients according to the method published by Bartko and Carpenter (Bartko and Carpenter Jr., 1976). The specific binding ratios for ^{123}I - β -CIT were compared using the Mann-Whitney *U* test. For 5-HTT binding ratios in the midbrain/pons region and in the left and right thalamus were assessed; for DAT binding the ratio in the striatum was assessed. Spearman rank correlation coefficients were calculated to assess correlations between specific binding ratios and LSAS scores. Two-tailed significance is reported throughout. Bonferroni correction for multiple comparisons (four regions of interest) yielded an adjusted *P* value of $p < 0.0125$.

Results

The intraclass correlation coefficients for the interrater and the intrarater reliability procedure for determining VOIs were between 0.86 and 0.99 (mean \pm SD, 0.95 ± 0.05) and 0.61 and 0.98

(mean, 0.81 ± 0.14), respectively. In one patient, only the 5-HTT uptake could be calculated, the last SPECT scan could not be reliably coregistered to the MRI scan because of motion artifacts.

The VOIs for the cerebellum were $104,208 \pm 16,211 \text{ mm}^3$ for patients and $93,943 \pm 11,445 \text{ mm}^3$ for controls. The VOIs for the midbrain/pons regions were $6,441 \pm 1,370 \text{ mm}^3$ for patients and $6,127 \pm 1,455 \text{ mm}^3$ for controls. The VOIs for the right thalamus were $3,962 \pm 855 \text{ mm}^3$ for patients and $4,544 \pm 1,678 \text{ mm}^3$ for controls, and the VOIs for the left thalamus were $4,051 \pm 914$ for patients and $4,610 \pm 686 \text{ mm}^3$ for controls. The VOIs for the right caudate were $3,142 \pm 519 \text{ mm}^3$ for patients and $2,933 \pm 608 \text{ mm}^3$ for controls, and the VOIs for the right putamen $2,064 \pm 407 \text{ mm}^3$ for patients and $1,990 \pm 497 \text{ mm}^3$ for controls. The VOIs for the left caudate were $2,899 \pm 598 \text{ mm}^3$ for patients and $3,181 \pm 573 \text{ mm}^3$ for controls, and the VOIs for the left putamen were $2,022 \pm 478 \text{ mm}^3$ for patients and $2,064 \pm 407 \text{ mm}^3$ for controls. There were no significant differences in the sizes of the delineated VOIs between patients and controls.

There were no significant differences in the normalized binding in the reference region between patients and controls; normalized cerebellar counts at four hours were 20.38 ± 3.70 in controls and 20.90 ± 4.12 in patients and at 22-24 hours the counts were 3.75 ± 1.15 in controls and 3.37 ± 1.02 in patients.

The Mann-Whitney U test revealed that the average binding ratio for 5-HTT in the left and right thalamus was significantly higher in patients than in matched healthy controls ($P = 0.001$) (Fig. 1).

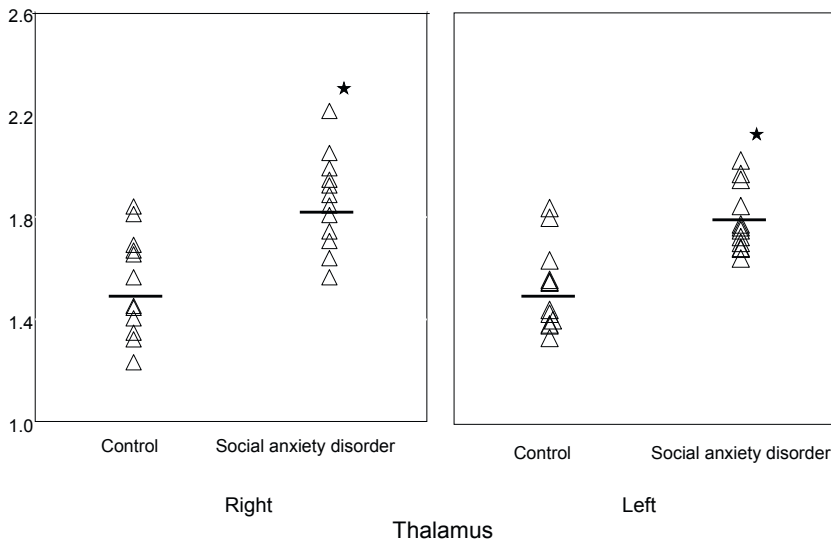


Figure 1. Binding ratios for 5-HTT in left and right thalamus of psychotropic medication-naïve patients with generalized social anxiety disorder ($N = 12$) and age- and sex-matched controls ($N = 12$) measured with ^{123}I - β -CIT SPECT. * $P = 0.001$; 2-tailed Mann-Whitney U test.

No significant differences were found in the midbrain/pons region. The average binding ratio for DAT in the striatum was significantly higher in patients than in matched controls ($P = 0.011$) (Fig. 2).

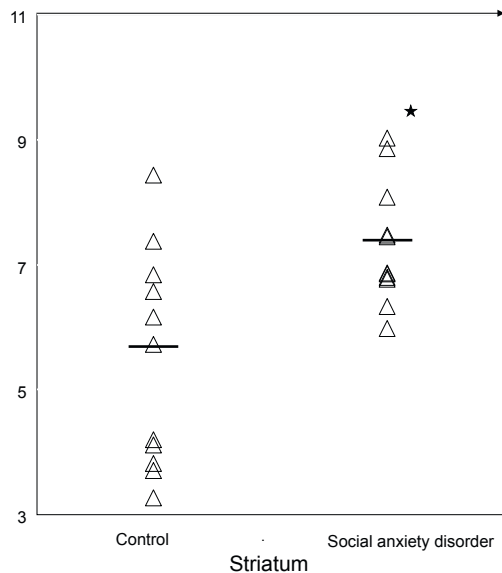


Figure 2. Binding ratios for the DAT in striatum of psychotropic medication-naïve patients with generalized social anxiety disorder (N = 12) and age- and sex-matched controls (N = 12) measured with ¹²³I-β-CIT SPECT. * P = 0.011; 2-tailed Mann-Whitney U test.

The binding ratios for 5-HTT and DAT in the regions of interest in patients and controls are summarized in Table 2. No significant correlations were found between LSAS score and DAT or 5-HTT binding potential in patients.

We performed an exploratory post-hoc delineation and analysis for right and left striatum and for left and right putamen and caudate. This analysis revealed that DAT binding in the right putamen was significant higher in patients than in matched healthy controls at a significance level uncorrected for multiple comparisons (P = 0.012).

Table 2 Average Binding Potentials for DAT and 5-HTT in Study Population.

VOI	Patients (N =12)	Controls (N=12)	P (Mann-Whitney U test)
5-HTT			
Left thalamus	1.79 ± 0.12	1.52 ± 0.65	0.001 *
Right thalamus	1.85 ± 0.19	1.53 ± 0.19	0.001 *
Midbrain/pons	0.94 ± 0.10	0.90 ± 0.19	0.713
DAT			
Striatum	7.30 ± 0.98	5.47 ± 1.37	0.011 *

* Value survived Bonferroni correction for multiple comparisons implying 4 regions of interest. Data are mean ± SD.

Discussion

We found significantly higher ^{123}I - β -CIT binding ratios in the left and right thalamus (specific for 5-HTT) and in the striatum (specific for DAT) of psychotropic medication-naïve patients with generalized social anxiety disorder with no comorbid diagnosis, relative to the findings in healthy controls pair wise matched for age, sex, and handedness. No abnormalities in binding ratios to the 5-HTT-rich midbrain/pons region were found. No significant correlations were found between 5-HTT and DAT binding ratios and scores on the symptom rating scale (LSAS).

To our knowledge this was the first study examining ^{123}I - β -CIT binding ratios both to 5-HTT-rich regions and to DAT-rich regions in patients with generalized social anxiety disorder. Our finding of an altered 5-HTT binding potential in the thalamus provides a direct indication that 5-HT has a role in the pathophysiology of generalized social anxiety disorder. Converging data have implicated a network of brain regions, including the prefrontal cortex, striatum, thalamus, and amygdale, in the pathophysiology of generalized social anxiety disorder (Stein et al., 2002; Mathew et al., 2001). Most regions of this putatively involved network in social anxiety disorder are densely innervated by serotonergic or dopaminergic neurons. 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 social anxiety disorder (Li et al., 2001).

Our finding of higher binding potentials of ^{123}I - β -CIT for 5-HTT in the thalamus of patients with social anxiety disorder can be interpreted as resulting from a decreased extracellular 5-HT concentration near the transporter (allowing ^{123}I - β -CIT to bind with higher density), from an elevated density of 5-HTT, or from a combination of both.

Decreased extracellular 5-HT levels in the brain of patients with social anxiety disorder would seem to be compatible with the fact that SSRIs are effective in social anxiety disorder (Ballenger et al., 1998). In line with this notion, it has been reported that repeated administration of SSRIs to healthy volunteers may increase social affiliation (Knutson et al., 1998). More recently, Argyropoulos et al. showed that reducing 5-HT availability in the brain through tryptophan depletion resulted in a significant increase in challenge-related anxiety in successfully treated patients with social anxiety disorder (Argyropoulos et al., 2004). The hypothesis of a decreased serotonergic transmission remains in contrast to other reports suggesting that increased 5-HT neurotransmission is anxiogenic. Harmer et al. recently reported an impaired recognition of fearful facial expression in female volunteers after tryptophan depletion whereas acute administration of the SSRI citalopram to healthy volunteers increased the recognition of fearful faces (Harmer et al., 2003a; Harmer et al., 2003b). Remarkably, SSRIs often display an acute anxiogenic effect that converts into anxiolysis on repeated administration. The mechanism responsible for this reversal in effect is unknown, but might be explained by adaptive changes (dampening) in the serotonergic system or in other more distal neuronal networks.

The heightened 5-HTT binding potential may also be the result of increased densities of 5-HTT in patients with social anxiety disorder, reflecting a higher homeostatic tone of the

serotonergic system (with concomitant lower densities of 5-HT receptors). This possibility would be in line with the results from Lanzenberger et al., who found reduced 5-HT_{1A} receptor levels in social anxiety disorder (Lanzenberger et al., 2007). Finally, the heightened 5-HTT binding potential may also be genetically determined. Arbelle et al recently reported an association between the 5-HTT promoter region 44-base-pair insertion/deletion and shyness in a non-clinical sample of second-grade children (Arbelle et al., 2003). The investigators reported a significant association between the long 5-HTT promoter region 44-base-pair insertion/deletion polymorphism and shyness in their sample. Children who were homozygote for the long allele, which has been shown to produce higher gene transcription and presumably a higher density of the 5-HTT, had significantly higher scores on the Shyness Scales (Murphy et al., 2001). In so far as shyness is an endophenotype for social anxiety disorder, the higher 5-HTT density may be interpreted as a risk factor for developing the disorder, which in turn may also explain our finding of a heightened 5-HTT binding potential. Unfortunately, the genetics of social anxiety disorder have not been adequately studied yet. Interestingly, a study by van Dyck et al. did not point at a direct association of higher central 5-HTT levels with the 5-HTT polymorphism but suggested a more complex relationship (Van Dyck et al., 2004).

The higher DAT binding potential in the striatum observed in this study is at variance with the data reported previously by Tiihonen et al., who found a decreased striatal dopamine binding potential in social anxiety disorder by using the same tracer (Tiihonen et al., 1997). The difference in outcome between the two studies cannot be readily explained. The most obvious differences between the two studies are our more accurate assessment of the VOIs by using MRI scan co-registration and the inclusion of psychotropic medication-naïve patients without comorbidity in the present study. Both studies, however, had a small number of subjects, a limitation that always bears a risk of false-positive outcomes. As discussed above, when interpreting the data of our study, one must consider that the interaction between radiotracer and transporter is determined by the amount of radiotracer, the amount of transporter and its affinity, and the amount of competing ligands, that is, endogenous dopamine. Thus, the present finding can be interpreted as either a lower level of extracellular dopamine or an elevated density of DAT in patients with social anxiety disorder or a combination of both.

By and large, previous studies examining the dopaminergic system in social anxiety disorder seem to point to a decreased dopaminergic activity. Schneier et al reported a lower ¹²³I-iodobenzamide binding potential for the dopamine D₂ receptors in patients with social anxiety disorder. The authors attributed this finding to a lower dopamine activity (Schneier et al., 2000). The lower binding potential, however, would also be reconcilable with either an enhanced dopaminergic activity or (transitory) high levels of dopamine near the receptors or an altered affinity of the receptor as was discussed by Mathew et al. (Mathew et al., 2001). Heightened dopaminergic activity may decrease the density or affinity of D₂ receptors and simultaneously upregulate the density of DAT, whereas high levels of dopamine may compete with ¹²³I-iodobenzamide for receptor binding. Data from animal models have shown that a heightened dopaminergic activity in the striatum during stress can decrease D₂ receptor density (Grant et al., 1998). In line with the

notion of an enhanced dopaminergic activity, Barnett et al. recently reported beneficial effects for the atypical antipsychotic olanzapine in patients with social anxiety disorder (Barnett et al., 2002). Taking together, these findings suggest that our observation of a heightened density of DAT in the striatum is probably best explained by an elevated dopaminergic transmission. Interestingly, a recent functional MRI study using an implicit learning task as a probe of striatal functioning, showed a reduced task-related activation of the striatum in patients with social anxiety disorder (Sareen et al., 2007). Although several studies have implicated the striatum in seasonal affective disorder, the involvement of specific striatal subregions has been less thoroughly examined. With our exploratory post-hoc analysis we found an increased DAT binding in the right putamen in seasonal affective disorder. However, this increase was significant at a level uncorrected for multiple comparisons, and the involvement of the putamen in seasonal affective disorder should be corroborated in other studies using other methodologies.

Clearly, the possible role of abnormalities in the dopaminergic and serotonergic systems needs to be further elucidated. Both dopamine (through D_1 and D_2 receptors) and serotonin (through 5-HT₂ receptors) are known to modulate the activity of excitatory (i.e., glutamate) and inhibitory (i.e., γ -aminobutyric acid) neurotransmitters in the striatum and related corticothalamolimbic circuitry. Data on the exact nature of these interactions are still inconclusive. Finally, based on the results of the present study it is not possible to dissect out whether the found dopaminergic and serotonergic abnormalities are causal or epiphenomenal to social anxiety disorder. In our study we found no significant correlations between scores on the clinical rating scale and abnormalities in the serotonergic and dopaminergic systems. In general, neuroimaging studies in psychiatry tend to find no or only weak correlations between the often heterogeneous symptomatology and neuroimaging measures. The previous ¹²³I- β -CIT study in social anxiety disorder also did not find correlations between binding ratios and symptomatology (Tiihonen et al., 1997). The absence of any correlation in our and the previous study may be due to the psychometric properties of the used clinical scale and the heterogeneity of social anxiety disorder as defined by the DSM-IV, but can also be interpreted as pointing to the fact that the phenomena of social anxiety disorder are not directly related to the found abnormalities. It is also important to note the overlap between the binding patterns in patients and controls, suggesting that the found abnormalities may perhaps be more related to vulnerability or personality traits. Another explanation may be that the found abnormalities are a consequence of having social anxiety disorder (i.e., a 'scar' hypothesis).

Our study had several strong points. The patients and controls were pair wise matched. Patients were psychotropic medication-naïve and had no comorbid diagnosis on Axis I, and most had not received prior psychotherapy. Furthermore, the SPECT data were analyzed using coregistered MRI scans, allowing for more precise determination of VOIs. There are some potential limitations to the present study. The sample size was relatively small and we used a limited number of VOIs. We visualized binding to the 5-HTT only at four hours after administration of the ligand. This time point for visualization, however, could have limited the possibility of finding further abnormalities at the level of 5-HTT, as was illustrated by the study of Willeit et al. on seasonal affective disorder (Willeit et al., 2000). In that study, 5-HTT was visualized at 4 hours after injection

of ^{123}I - β -CIT and also at 24 hours, when a pseudoequilibrium state is reached. Differences were found only in the SPECT acquisitions at 24 hours after the injection. We followed the method described by Kuikka et al. and used paroxetine 20 mg to completely displace the ^{123}I - β -CIT from 5-HTT (Kuikka et al., 1995). Administration of paroxetine could potentially lead to an increase in symptoms of (social) anxiety, but such an increase (mild) was reported by only one patient.

Finally, although SPECT is easier to use, is less expensive, and has a higher safety index than PET, it also uses semi-quantitative techniques and has a poorer anatomical resolution.

Conclusion

Our data provide direct evidence for the involvement of both the dopaminergic and the serotonergic systems in the pathophysiology of social anxiety disorders. These findings need to be replicated and further explored in studies examining the effect of pharmacotherapy and psychotherapy on both the serotonergic and the dopaminergic transporter and receptor-binding capacities in generalized social anxiety disorder.

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References

- American Psychiatric Association (1994). *Diagnostic and statistical manual of mental disorders DSM-IV*. American Psychiatric Press, Washington, DC.
- Arbelle, S., Benjamin, J., Golin, M., Kremer, I., Belmaker, R. H., and Ebstein, R. P. (2003). Relation of shyness in grade school children to the genotype for the long form of the serotonin transporter promoter region polymorphism. *Am J Psychiatry*, 160, 671-676.
- Argyropoulos, S. V., Hood, S. D., Adrover, M., Bell, C. J., Rich, A. S., Nash, J. R. et al. (2004). Tryptophan depletion reverses the therapeutic effect of selective serotonin reuptake inhibitors in social anxiety disorder. *Biol Psychiatry*, 56, 503-509.
- Ballenger, J. C., Davidson, J. R., Lecrubier, Y., Nutt, D. J., Bobes, J., Beidel, D. C. et al. (1998). Consensus statement on social anxiety disorder from the International Consensus Group on Depression and Anxiety. *J Clin Psychiatry*, 59 Suppl 17, 54-60.
- Barnett, S. D., Kramer, M. L., Casat, C. D., Connor, K. M., and Davidson, J. R. (2002). Efficacy of olanzapine in social anxiety disorder: a pilot study. *J Psychopharmacol*, 16, 365-368.
- Bartko, J. J. and Carpenter, W. T., Jr. (1976). On the methods and theory of reliability. *J Nerv Ment Dis*, 163, 307-317.
- Collins, D. L., Neelin, P., Peters, T. M., and Evans, A. C. (1994). Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. *J.Comput.Assist.Tomogr.*, 18, 192-205.
- De Win, M. M., Habraken, J. B., Reneman, L., van den, B. W., den Heeten, G. J., and Booij, J. (2005). Validation of [(123)I]beta-CIT SPECT to assess serotonin transporters in vivo in humans: a double-blind, placebo-controlled, crossover study with the selective serotonin reuptake inhibitor citalopram. *Neuropsychopharmacology*, 30, 996-1005.
- Grant, K. A., Shively, C. A., Nader, M. A., Ehrenkaufer, R. L., Line, S. W., Morton, T. E. et al. (1998). Effect of social status on striatal dopamine D-2 receptor binding characteristics in cynomolgus monkeys assessed with positron emission tomography. *Synapse*, 29, 80-83.
- Hamilton, M. (1967). Development of a rating scale for primary depressive illness. *Br.J.Soc.Clin.Psychol.*, 6, 278-296.
- Harmer, C. J., Bhagwagar, Z., Perrett, D. I., Vollm, B. A., Cowen, P. J., and Goodwin, G. M. (2003b). Acute SSRI administration affects the processing of social cues in healthy volunteers. *Neuropsychopharmacology*, 28, 148-152.
- Harmer, C. J., Rogers, R. D., Tunbridge, E., Cowen, P. J., and Goodwin, G. M. (2003a). Tryptophan depletion decreases the recognition of fear in female volunteers. *Psychopharmacology (Berl)*, 167, 411-417.
- Heimberg, R. G., Horner, K. J., Juster, H. R., Safren, S. A., Brown, E. J., Schneier, F. R. et al. (1999). Psychometric properties of the Liebowitz Social Anxiety Scale. *Psychol Med*, 29, 199-212.
- Hollander, E., Kwon, J., Weiller, F., Cohen, L., Stein, D. J., DeCaria, C. et al. (1998). Serotonergic function in social phobia: comparison to normal control and obsessive-compulsive disorder subjects. *Psychiatry Res*, 79, 213-217.
- Johnson, M. R., Lydiard, R. B., Zealberg, J. J., Fossey, M. D., and Ballenger, J. C. (1994). Plasma and CSF HVA levels in panic patients with comorbid social phobia. *Biol Psychiatry*, 36, 425-427.
- Knutson, B., Wolkowitz, O. M., Cole, S. W., Chan, T., Moore, E. A., Johnson, R. C. et al. (1998). Selective alteration of personality and social behavior by serotonergic intervention. *Am J Psychiatry*, 155, 373-379.

- Kuikka, J. T., Tiihonen, J., Bergstrom, K. A., Karhu, J., Hartikainen, P., Viinamaki, H. et al. (1995). Imaging of serotonin and dopamine transporters in the living human brain. *Eur.J.Nucl.Med.*, 22, 346-350.
- Lanzenberger, R. R., Mitterhauser, M., Spindelegger, C., Wadsak, W., Klein, N., Mien, L. K. et al. (2007). Reduced Serotonin-1A Receptor Binding in Social Anxiety Disorder. *Biol.Psychiatry*, 61, 1081-1089.
- Laruelle, M., Wallace, E., Seibyl, J. P., Baldwin, R. M., Zea-Ponce, Y., Zoghbi, S. S. et al. (1994). Graphical, kinetic, and equilibrium analyses of in vivo [123I] beta-CIT binding to dopamine transporters in healthy human subjects. *J.Cereb.Blood Flow Metab*, 14, 982-994.
- Li, D., Chokka, P., and Tibbo, P. (2001). Toward an integrative understanding of social phobia. *J Psychiatry Neurosci*, 26, 190-202.
- MacDonald D.J. (1993). *Register: A users manual. Technical report*. Montreal: McGill University.
- MacDonald D.J. (1996). *Display: A Users Manual*. Montreal: McGill University.
- Mathew, S. J., Coplan, J. D., and Gorman, J. M. (2001). Neurobiological mechanisms of social anxiety disorder. *Am J Psychiatry*, 158, 1558-1567.
- Meyer, J. H., Wilson, A. A., Ginovart, N., Goulding, V., Hussey, D., Hood, K. et al. (2001). Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [(11)C]DASB PET imaging study. *Am J Psychiatry*, 158, 1843-1849.
- Murphy, D. L., Li, Q., Engel, S., Wichems, C., Andrews, A., Lesch, K. P. et al. (2001). Genetic perspectives on the serotonin transporter. *Brain Res Bull*, 56, 487-494.
- Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, 9, 97-113.
- Pirker, W., Asenbaum, S., Kasper, S., Walter, H., Angelberger, P., Koch, G. et al. (1995). beta-CIT SPECT demonstrates blockade of 5HT-uptake sites by citalopram in the human brain in vivo. *J Neural Transm.Gen Sect.*, 100, 247-256.
- Sareen, J., Campbell, D. W., Leslie, W. D., Maliszka, K. L., Stein, M. B., Paulus, M. P. et al. (2007). Striatal function in generalized social phobia: a functional magnetic resonance imaging study. *Biol.Psychiatry*, 61, 396-404
- Schneier, F. R., Liebowitz, M. R., Abi-Dargham, A., Zea-Ponce, Y., Lin, S. H., and Laruelle, M. (2000). Low dopamine D(2) receptor binding potential in social phobia. *Am J Psychiatry*, 157, 457-459.
- Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E. et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J.Clin.Psychiatry*, 59 Suppl 20, 22-33.
- Stein, D. J., Westenberg, H. G., and Liebowitz, M. R. (2002). Social anxiety disorder and generalized anxiety disorder: serotonergic and dopaminergic neurocircuitry. *J Clin Psychiatry*, 63 Suppl 6, 12-19.
- Stein, M. B., Heuser, I. J., Juncos, J. L., and Uhde, T. W. (1990). Anxiety disorders in patients with Parkinson's disease. *Am J Psychiatry*, 147, 217-220.
- Tancer, M. E., Mailman, R. B., Stein, M. B., Mason, G. A., Carson, S. W., and Golden, R. N. (1994). Neuroendocrine responsivity to monoaminergic system probes in generalized social phobia. *Anxiety*, 1, 216-223.
- Tauscher, J., Pirker, W., Willeit, M., de Zwaan, M., Bailer, U., Neumeister, A. et al. (2001). [123I] beta-CIT and single photon emission computed tomography reveal reduced brain serotonin transporter availability in bulimia nervosa. *Biol.Psychiatry*, 49, 326-332.

- Tiihonen, J., Kuikka, J., Bergstrom, K., Lepola, U., Koponen, H., and Leinonen, E. (1997). Dopamine reuptake site densities in patients with social phobia. *Am.J.Psychiatry*, 154, 239-242.
- Van der Wee, N. J., Stevens, H., Hardeman, J. A., Mandl, R. C., Denys, D. A., van Megen, H. J. et al. (2004). Enhanced dopamine transporter density in psychotropic-naïve patients with obsessive-compulsive disorder shown by [123I](Sareen et al., 2007b)-CIT SPECT. *Am J Psychiatry*, 161, 2201-2206.
- Van Dyck, C. H., Malison, R. T., Staley, J. K. et al. (2004). Central serotonin transporter availability measured with [123I]beta-CIT SPECT in relation to serotonin transporter genotype. *Am J Psychiatry*, 161, 525-31.
- Westenberg, H. G. (1998). The nature of social anxiety disorder. *J Clin Psychiatry*, 59 Suppl 17, 20-26.
- Willeit, M., Praschak-Rieder, N., Neumeister, A., Pirker, W., Asenbaum, S., Vitouch, O. et al. (2000). [123I]-beta-CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. *Biol.Psychiatry*, 47, 482-489.

Mirtazapine in social anxiety disorder: a pilot study

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Abstract

Fourteen patients with social anxiety disorder (generalized type), according to DSM-IV criteria, were treated with mirtazapine 30 mg for 12 weeks. Twelve patients completed the study. Two patients (14.3%) dropped out due to side-effects. Generally, mirtazapine was well tolerated. Five out of twelve 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.

Introduction

Social anxiety disorder (SAD), or social phobia, is one of the most common, but still underrecognized, psychiatric disorders. The majority of the epidemiological studies found lifetime prevalence rates of between 1.7% and 16.1%, using DSM-III, or -IV criteria (Brunello et al, 2000). An epidemiological survey found lifetime prevalence rates for the generalized type and the specific type of 5.9% and 7.0%, respectively (Stein and Kean, 2000). The essential feature of SAD is the fear of being negatively evaluated by others, resulting in avoidance of social situations or marked distress. Exposure to the feared situation provokes an immediate anxiety response, with symptoms such as trembling, blushing and sweating, and may even take the form of a situationally predisposed panic attack. Social anxiety and avoidance interferes significantly with the person's daily routine, social or occupational functioning. Treatment options for SAD are cognitive behavioural therapy and drug treatment.

The selective serotonin reuptake inhibitors (SSRIs) are the firstline drug treatment for SAD. Other treatment options include monoamine oxidase inhibitors (MAOIs) and benzodiazepines (Westenberg, 1999a). Information on other antidepressants, such as venlafaxine (Van Vliet et al., 1997; Altamura et al, 1999) and clomipramine (Van Vliet and Westenberg, 1999), suggest that they might also be effective in SAD, but data from controlled studies are lacking. Mirtazapine is an antidepressant with a novel mechanism of action. It has shown to block the α_2 -adrenergic autoreceptors, resulting in a stimulation of both noradrenergic and serotonergic pathways. In addition, mirtazapine also blocks the 5-HT₂ and 5-HT₃ receptors. By virtue of its antihistaminergic properties, sedation and weight gain may occur (De Boer, 1996).

Several small open label studies with mirtazapine have been performed in anxiety disorders. These studies in anxiety states (Sitsen and Moors, 1994), panic disorder (Carpenter et al, 1999), post-traumatic stress disorder (Connor et al, 1999), major depression with comorbid generalized anxiety disorder (Goodnick et al, 1999) and major depression with anxiety symptoms (Fawcett and Barkin, 1998) suggest mirtazapine has anxiolytic properties.

The aim of the present study was to assess the possible efficacy of mirtazapine in patients suffering from SAD and to evaluate whether large controlled studies with this drug are warranted.

Methods

Subjects

The present study was performed in the outpatient clinic of the University Medical Center Utrecht, the Netherlands. Patients meeting DSM-IV criteria for SAD, generalized type, who were aged 18-65 years, were included in the study. Patients with other axis I disorders, and pregnant or nursing women were excluded.

Study design

Subjects were treated with mirtazapine, 30 mg a day, fixed dose, for 12 weeks. They were evaluated at baseline, and at weeks 4 and 12. Responders were treated for another 12 weeks and evaluated at week 24. None of the patients had used psychotropic medication other than incidental use of oxazepam for more than a year prior to the study. Concomitant psychotropic medication was not allowed during the course of the study, except for oxazepam 10 mg, with a maximum of 20 mg a day.

The primary outcome measure was the Liebowitz Social Anxiety Scale (LSAS), comprising 24 items, in which anxiety and avoidance are rated in different social situations on a 0 – 3 points scale.

The other efficacy measures were the Clinical Global Improvement scale (CGI), and the Hamilton Depression Rating Scale (HDRS). The LSAS, and the HDRS were performed at baseline, LSAS and CGI were performed at week 4, and the LSAS CGI and HDRS were performed at week 12. Patients with a reduction of 40% or more on the LSAS total score and a CGI score ‘improved’ (2) or ‘much improved’ (1) were considered responders to treatment. Information on side-effects was collected by open questioning.

Statistical analysis

Multivariate analysis of variance with repeated measures (ANOVA) was used to evaluate the LSAS score in completers. Student’s paired t-test was used to determine significant differences between baseline and endpoint on the LSAS and HDRS.

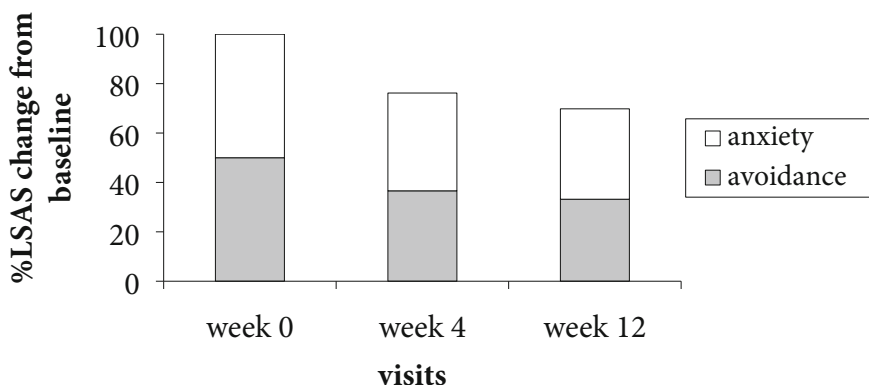


Figure 1. The mean Liebowitz Social Anxiety Scale (LSAS) total score of all patients (n=14), divided in an anxiety and an avoidance subscore, is shown as percent change from baseline. Statistical analysis revealed a significant change in anxiety and in avoidance subscores over time ($P<0.005$, d.f.=2,12, $F=8.85$; $P<0.001$, d.f.=2,12, $F=15.13$). The changes from baseline were already statistically significant as of week 4 ($P<0.005$, $t=4.00$; $P<0.001$, $t=7.03$). Analysis with last observation carried forward also showed a significant change in anxiety and avoidance subscores from baseline to week 12 ($P<0.01$, $t=3.14$; $P<0.005$, $t=3.98$)

Results

Fourteen patients (11 males and three females), aged 23-44 years, who were suffering from the generalized type of SAD, were included in this study. The mean age was 35.5 years. The mean

age of onset of SAD was 13.4 years, and the mean duration of illness was 22 years. Previous drug treatment with antidepressant medication, mostly SSRIs, was reported in 28.6% of patients. Previous cognitive behavioural therapy was applied in 35.7% of the patients, and 64.3% had a family history of SAD. Mirtazapine was well tolerated in the majority of the patients. The most frequently reported side-effects were sedation and weight gain. Other side-effects reported were a dry mouth, muscle cramps, dizziness, insomnia and restless sleep. Two patients used oxazepam 10 mg occasionally. Two patients dropped out after 4 weeks due to weight gain and sedation. All responders to treatment continued taking mirtazapine after completion of the study.

The mean LSAS total score at baseline was 72.6 ± 5.11 , and decreased to 48.3 ± 8.18 at week 12. The change in mean LSAS score is shown in figure 1. Statistical analysis revealed a significant change in LSAS score over time ($P < 0.001$, d.f.=2,12, $F=12.33$), and this was already statistically significant by week 4 ($P < 0.001$, $t=5.25$). Analysis with last observation carried forward (LOCF) also showed a significant change in LSAS score from baseline to week 12 ($P < 0.05$, $t=3.59$). Five out of 12 (41.7%) completers were classified as responders to treatment, with a mean LSAS total score of 67.6 at baseline ($n=5$), and 24.5 at week 12. No further improvement was seen at week 24. The mean HDRS score decreased from 7.4 at baseline to 5.0 at week 12 ($P < 0.05$, $t=3.62$).

Discussion

This open pilot study suggests that mirtazapine is a clinically effective treatment for SAD of the generalized type. Mirtazapine was generally well tolerated. Using a stringent criterion for response, 41.7% of the patients were classified as responders. Because no comorbidity, such as depression, was allowed in this study, these effects of mirtazapine appear to be specific, and cannot be attributed to the antidepressant effect of the drug.

The present study is the first study to describe the effects of mirtazapine in SAD. The pharmacological properties of mirtazapine are quite different from SSRIs, the treatment of choice for SAD thus far. Mirtazapine is an α_2 -adrenergic receptor antagonist with additional antagonistic activity at the 5-HT₂ and 5-HT₃ receptors. Unlike SSRIs, mirtazapine has no direct stimulatory effects on the serotonergic system. According to its different mechanism of action, mirtazapine has a side-effect profile that completely differs from SSRIs. Mirtazapine is devoid of side-effects common to all SSRIs, such as nausea, sleep disturbances and sexual dysfunction, which might influence patient's compliance and lead to treatment discontinuation (Westenberg and Den Boer, 2001). Mirtazapine lacks these side-effects because it blocks the 5-HT₂ and 5-HT₃ receptors, and even shows sleep improvement (Thase et al, 2001). In contrast to SSRIs, the most prominent side-effects of mirtazapine are daytime sedation and weight gain (De Boer, 1996). The efficacy of SSRIs in SAD is well documented. Studies with SSRIs using a similar response criterion as that in the present study, generally show an efficacy of between 50% and 60%, which is slightly higher than the response of 42% seen in the present study (Westenberg, 1999b). However, it should be noted that this was a fixed dose study and that no information is available on the optimal dose of mirtazapine in SAD. In conclusion, double-

blind research is clearly warranted to confirm the efficacy of mirtazapine in SAD. By virtue of its different pharmacological profile, mirtazapine might be an alternative treatment option for those patients who can not tolerate, or are unresponsive to SSRIs.

References

- Altamura, A.C., Piol, R., Vitto, M., Mannu, P. (1999). Venlafaxine in social phobia: a study in selective serotonin reuptake non-responders. *Int. Clin. Psychopharmacol.* 14:239-245.
- Brunello, N., Den Boer, J.A., Judd, L.L., Kasper, S., Kelsey, J.E., Lader, M., Lecrubier, Y., Lepine, J.P., Lydiard, R.B., Mendlewicz, J., Montgomery, S.A., Racagni, G., Stein, M.B., Wittchen, H.U. (2000). Social phobia: Diagnosis and epidemiology, neurobiology and pharmacology, comorbidity and treatment. *J. Affect. Disord.* 60: 61-74.
- Carpenter, L.L., Leon, Z., Yasmin, S., Price, L.H. (1999). Clinical experience with mirtazapine in the treatment of panic disorder. *Ann. Clin. Psychiatry* 11:81-86.
- Connor, K.M., Davidson, J.R., Weisler, R.H., Ahearn, E. (1999). A pilot study of mirtazapine in posttraumatic stress disorder. *Int. Clin. Psychopharmacol.* 14:29-31.
- De Boer, Th. (1996) The pharmacological profile of mirtazapine. *J. Clin. Psychiatry* 57(suppl 4):19-25.
- Fawcett, J., Barkin, R.L. (1998). A meta-analysis of eight randomized, double-blind, controlled clinical trials of mirtazapine for the treatment of patients with major depression and symptoms of anxiety. *J. Clin. Psychiatry* 59:123-127.
- Goodnick, P.J., Puig, A., DeVane, C.L., Freund, B.V. (1999). Mirtazapine in major depression with comorbid generalized anxiety disorder. *J. Clin. Psychiatry* 60:446-448.
- Sitsen, J.M.A., Moors, J. (1994). Mirtazapine, a novel antidepressant, in the treatment of anxiety symptoms. *Drug Invest.* 8:339-344.
- Stein, M.B., Kean, Y.M. (2000). Disability and the quality of life in social phobia: Epidemiologic findings. *Am. J. Psychiatry* 157:1606-1613.
- Thase, M.E., Howland, R.H., Friedman, E.S. (2001). Onset of action of selective and multi-action antidepressants. In: Focus on psychiatry: Antidepressants: Selectivity or multiplicity (Westenberg, H.G.M., Den Boer, J.A. eds), Amsterdam, The Netherlands, Benecke N.I., pp 101-116.
- Van Vliet, I.M., Westenberg, H.G.M. (1999). Pharmacotherapy of social anxiety disorder. In: Focus on psychiatry: Social Anxiety Disorder (Westenberg, H.G.M., Den Boer, J.A., eds), Amsterdam, The Netherlands: Syn-Thesis Publishers, pp 157-174.
- Van Vliet, I.M., Westenberg, H.G.M., Van Megen, H.J.G.M. (1997). Clinical effects of venlafaxine in social phobia. *Eur. Neuropsychopharmacol.* 8(suppl 2):s258.
- Westenberg, H.G.M. (1999a). Facing the challenge of social anxiety disorder. *Eur. Neuropsychopharmacol.* 9(suppl 3):S93-S99.
- Westenberg, H.G.M. (1999b). Selective serotonin reuptake inhibitors in social anxiety disorder. In: Focus on psychiatry: Social anxiety disorder (Westenberg, H.G.M., Den Boer, J.A., eds), Amsterdam, The Netherlands: Syn-Thesis Publishers, pp175-90.
- Westenberg, H.G.M., Den Boer, J.A. (2001). Pharmacology of antidepressants: Selectivity or multiplicity. In: Focus on psychiatry: Antidepressants: Selectivity or multiplicity (Westenberg, H.G.M., Den Boer, J.A., eds), Amsterdam, The Netherlands, Benecke N.I., pp 31-42.

Elevated alpha-amylase but not cortisol in generalized social anxiety disorder

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Abstract

Stress system dysregulation is thought to increase the risk for anxiety disorders. Here we describe both hypothalamic pituitary adrenal (HPA) axis and autonomic nervous system (ANS) activity in basal non-challenging conditions and after 0.5 mg dexamethasone in generalized social anxiety disorder (gSAD) patients. To ensure stress-free sampling we collected saliva 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 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. Cortisol and sAA were analyzed with mixed models. Additional analyses were done with paired *t*-tests. Apart from the assessments in the morning, gSAD patients had significantly higher diurnal and post-dexamethasone 16.00h 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 as compared to the HPA-axis, in line with the observed hyperarousal in gSAD.

Introduction

Social anxiety disorder (SAD; also known as social phobia) is one of the most common anxiety disorders (Stein, 2006). It is characterised by the fear of scrutiny by others. In specific SAD (sSAD) one or two situations are feared, e.g. speaking in public, while in generalized SAD (gSAD) most social situations are involved (American Psychiatric Association, 2000). The anxiety in gSAD is often accompanied by hyperarousal, such as increased heart rate, trembling, blushing and sweating. These clinical observations suggest an involvement of the stress system (De Kloet et al., 2005; Goldstein, 2003). However, whether the stress system is involved in gSAD is an unresolved question. Moreover, it is unclear under which conditions (basal versus challenge) and which parts of the stress system (autonomic nervous system (ANS) or the hypothalamic pituitary adrenal (HPA) axis) are involved. In gSAD, these systems have never been studied in concert, instead previous studies aimed to elucidate the role of these systems separately.

The function of the HPA-axis in SAD has been investigated by using cortisol levels as biological marker. In basal conditions no differences were found between gSAD patients and controls with respect to 24-hour urine cortisol levels (Potts et al., 1991; Uhde et al., 1994). Moreover, diurnal saliva cortisol levels of adolescent girls with SAD were comparable with those of healthy controls (Martel et al., 1999). The last study can be criticized since no distinction was made between subtypes of SAD. With some, but not all psychological challenges, HPA-axis dysfunction was reported. In one study gSAD patients had a significantly larger cortisol response to the Trier Social Stress Test than controls (Condren et al., 2002). Another study found a more outspoken dichotomous cortisol response after a stress challenge in gSAD compared to the control group: the cortisol responders in the group of gSAD patients had higher cortisol levels after the stress task than the cortisol responders in the control group, the cortisol non-responders in the gSAD group had lower cortisol levels than the non-responders in the control group (Furlan et al., 2001). However, in the study with adolescent girls mentioned above, Martel et al. (Martel et al., 1999) did not find any difference in cortisol responses between SAD and controls after a public speaking challenge. One study on the effects of a pharmacological challenge with dexamethasone in gSAD was published, reporting no differences between gSAD patients and controls in 24-hour urine cortisol levels (Uhde et al., 1994).

The function of the ANS has been studied in various ways. Stein and colleagues found higher noradrenaline levels in gSAD patients in one study, but could not replicate this finding in another (Stein et al., 1992, 1994). One study reported on higher baseline heart rate and blood pressure in gSAD patients compared to controls (Bouwer and Stein, 1998), whereas other studies did not (Grossman et al., 2001; Laederach-Hofmann et al., 2002; Gerlach et al., 2003).

A major drawback of autonomic measures, especially plasma (nor)adrenaline obtained by venipuncture, is the stress accompanying the sampling. Furthermore, heart rate and blood pressure are easily influenced by many factors, e.g. posture.

Recently, salivary alpha-amylase (sAA) has been proposed to reflect ANS activity (Chatterton, Jr. et al., 1996; Granger et al., 2007). In basal conditions in healthy volunteers, sAA

activity shows a diurnal profile with a decrease during the first 60 minutes after awakening and an increase during the rest of the day (Rohleder et al., 2004; Nater et al., 2007). sAA levels were relatively independent of several possible confounders like gender, body mass index (BMI), activity level, smoking, eating and drinking but significantly associated with chronic stress and stress reactivity in healthy volunteers (Nater et al., 2007).

Psychological and physiological challenges were followed by increases in sAA (Gilman et al., 1979; Bosch et al., 1996; Chatterton, Jr. et al., 1996; Chatterton, Jr et al., 1997; Rohleder et al., 2004; Rohleder et al., 2006; Nater et al., 2005; Nater et al., 2006; Kivlighan and Granger, 2006), although most studies failed to find significant correlations between adrenaline and noradrenaline, peripheral markers of the ANS, and sAA (Rohleder et al., 2004; Nater et al., 2006) indicating that stress-dependent sAA increases reflect changes of the ANS in general, not catecholamine increases. Furthermore, two pharmacological challenges provided direct evidence that sAA measures ANS activity. Propranolol (a non-selective β -blocker), combined with a stressful task, resulted in lowering of sAA, showing the sensitivity of sAA to changes in adrenergic activity (Van Stegeren et al., 2006). Similarly, a yohimbine (an α_2 -receptor antagonist) challenge induced not only increases of peripheral noradrenaline levels but also increases of sAA levels compared to placebo. No correlations were found between adrenaline, noradrenaline and sAA, indicating that sAA reflects central noradrenaline release in stead of peripheral noradrenaline secretion (Ehlert et al., 2006). More research on sAA is discussed in the review of Granger et al. (2007).

The question remains whether there is a difference in basal autonomic activity in gSAD as compared to controls. Furthermore, we aimed to investigate the ANS and the HPA-axis in concert, since the specific balance between these stress systems, as was shown in experimental animal research, is often overlooked (Goldstein, 2003). Considering sAA as a measure of autonomic activity together with stress-free sampling of saliva stimulated us to use sAA to test basal autonomic activity in gSAD.

Here we report the awakening and diurnal rhythm of both cortisol and sAA, in basal conditions. In order to also measure the feedback sensitivity of the HPA-axis, we added a low-dose dexamethasone suppression test with 0.5 mg (Gaab et al., 2002), which is more sensitive to detect differences between different groups than higher dosages. We expected, based on former research, not to find differences in HPA-axis function in gSAD as compared to controls. However, we hypothesized that the ANS, as measured using sAA, would show hyperactivity in gSAD during the day.

Methods

Subjects

Forty-three patients with gSAD and 43 age (± 5 years) and gender matched healthy controls participated in this study. No life-time psychiatric comorbidity or clinically significant medical disorders, such as endocrinological disorders, were allowed. Patients were not currently treated for gSAD. Subjects were excluded in the case of alcohol consumption of over three units a day, using drugs of abuse or smoking more than 20 cigarettes a day. Use of psychotropic medication (including β -

blocking agents) had to be stopped at least 14 days before the trial, with the exception of oxazepam (half life 4 to 15 hours) with a maximum dosage of 30 mg a day and only when used sporadically on an 'as needed' basis. It was allowed to be used up to 24 hours before the test days. No oral glucocorticoids were allowed in 6 months prior to the study. Because of the influence of estradiol on the HPA-axis, women were tested during the follicular phase of the menstrual cycle and women using oral contraceptives in the stop week. Women were asked about their menstruations in order to assess perimenopause (period of irregular menstruations before menopause) or menopause (last menstruation over a year ago). In case of any doubt, hormonal assessments were performed and discussed with a gynaecologist. Perimenopausal women were excluded, postmenopausal women were included.

Procedures

The protocol was conducted in accordance with the declaration of Helsinki and approved by the Medical Ethical Committee of the Leiden University Medical Center. Patients and controls were recruited by advertisements and interviews in local papers. Subjects contacted us and the investigator screened them by telephone on in- and exclusion criteria. If the criteria were met, the possible subjects were invited to visit our hospital. The possible controls were invited for a screening visit and received an information letter regarding the study by mail. In the case of suspected gSAD, patients were first invited for a diagnostic interview, then received the information letter and when eligible they were invited for the screening visit. Screening was performed within a 2-week period prior to the study. Before starting the screening, after complete description of the study, subjects signed the informed consent form. At the screening visit, DSM-IV diagnosis was confirmed and comorbidity was checked with the MINI Plus 5.0.0 (Van Vliet and De Beurs, 2007). Subjects were asked for their medical history and physical symptoms of illness. Then a physical examination was done, and on indication a laboratory investigation. The Liebowitz Social Anxiety Scale (LSAS) and the 17-points Hamilton Rating Scale for Depression (HRDS) were used in both groups to measure symptom severity (Liebowitz, 1987; Hamilton, 1960). Subjects with a HRDS score over 16 were excluded. A summary of the Structured Trauma Interview was used to make an inventory of experienced traumatic events (Draijer, 1989), because trauma might influence the HPA-axis even in people without PTSD.

In- and exclusion criteria were checked. After planning the two test days, salivettes and practical instructions were given.

The saliva samples were collected by the subjects at home during two non-working days. Patients and controls were specifically requested not to expose themselves to social situations or strenuous exercise during the test days, in order to measure stress markers in a basal, non-stressed condition. On test day I, samples were taken immediately after awakening, 30 minutes, 45 minutes and 60 minutes after awakening, at 1100 h, 1500 h, 1900 h, and 2200 h. At 2300 h they ingested a tablet of 0.5 mg dexamethasone. On test day II (the following day) subjects were asked to collect saliva samples at 900 h and 1600 h. After these test days subjects returned the salivettes through mail, and at arrival in our clinic the samples were frozen in -80 °C.

We measured the presence of dexamethasone in saliva in order to check on the reliability of the dexamethasone suppression test (DST) and to have an impression of the compliance of the subjects in both groups.

Instructions for saliva sampling

Saliva was obtained using salivette collecting devices, containing a cotton wad (Sarstedt, Rommelsdorf, Germany). For each sample subjects were asked to chew on this wad until the cotton was saturated with saliva, to put it back into the tube and then to keep it in the refrigerator. In order to improve compliance and to detect deviations from the protocol subjects were told: (1) to strictly follow the procedures and the time schedule for saliva sampling to obtain valuable data; (2) to record sample times and activities in the hour before sampling; (3) that we would be able to check compliance by looking at their cortisol curves. For the awakening sample, subjects were instructed to start saliva sampling immediately at awakening. Subjects were told to complete the early morning samples (awakening and 30 minutes, 45 minutes and 60 minutes afterwards) before brushing their teeth and having breakfast to avoid contamination of saliva with blood, food or drinks. They also were requested not to eat fruit or drink fruit juices and to thoroughly rinse their mouth with tap water before sampling saliva to avoid contamination.

Laboratory analysis of sAA and cortisol

The determination of cortisol in saliva was performed with a competitive electrochemiluminescence immunoassay ECLIA using a Modular Analytics E1 70 immunoassay analyzer from Roche Diagnostics (Mannheim, Germany). The sample volume was 20 μ L. The detection limit was 2 nmol/l (Van Aken et al., 2003).

The determination of sAA was performed with an enzymatic colorimetric assay using the maltoheptaoside (EPS) substrate on a P-module clinical chemistry analyzer (Roche, Mannheim, Germany) in 400-fold diluted saliva samples. The detection limit was 3 U/l (Lorentz, 1998).

The dexamethasone concentration was measured by a home-made radioimmunoassay (Department of Clinical Chemistry, Leiden University Medical Center, J. van Pelt). Dexamethasone was extracted from saliva and then analyzed by radioimmunoassay, which was performed with the antidexamethasone antibody from IgG Corporation (Nashville, Tennessee). The protocol from IgG Corporation was slightly modified. The lower limit of detection was 50 pmol/l (17.5 ng/l) and the reported cross-reactivity for cortisol is 0.04% (Weijtens et al., 1997).

Statistics

Outliers in cortisol levels were removed when they were above 50 nmol/l and higher than the mean + 3 S.D. Outliers in sAA levels were removed when they were above the mean + 3 S.D. The missing data were interpolated linearly. This was not possible for missing data on time points 1, 8, 9, or 10, because they were at the end or beginning of the day. Missing data on time point 1 and 8 were replaced by those of time point 2 and 7, respectively. Missing data on time point 9 and 10 the data were recorded as missing.

1. For evaluation of cortisol and sAA levels, Area Under the Curve with respect to ground (AUCg) values were first calculated for each subject separately and relative to the baseline value, for both the awakening cycle as well as for diurnal cortisol (11-22 h). We used the following formula for the calculation of the AUCg awakening : $((\text{value}_{t_1} + \text{value}_{t_2}) / 2) \times (t_2 - t_1)) + ((\text{value}_{t_2} + \text{value}_{t_3}) / 2) \times (t_3 - t_2)) + ((\text{value}_{t_3} + \text{value}_{t_4}) / 2) \times (t_4 - t_3))$; and the corresponding formula for the diurnal AUCg: $((\text{value}_{t_5} + \text{value}_{t_6}) / 2) \times (t_6 - t_5)) + ((\text{value}_{t_6} + \text{value}_{t_7}) / 2) \times (t_7 - t_6)) + ((\text{value}_{t_7} + \text{value}_{t_8}) / 2) \times (t_8 - t_7))$.

Subsequently, the thus summarized data were evaluated via mixed modeling, in order to take the paired nature of the data within matched pairs into account. The model is formulated as $Y_{ij} = \alpha_i + \beta X_{ij} + \gamma_1 CF_{1(ij)} + \dots + \gamma_k CF_{k(ij)} + \xi_{ij}$ where Y_{ij} are the modeled responses on the observed pairs, with $i=1, \dots, 43$ indicating the pair and $j=1,2$ denoting the case and control status respectively of each of two observations within each i^{th} pair. We define $X_{ij}=1$ for $j=2$ and zero otherwise, such that β denotes the effect of substantive interest, contrasting cases from controls. The term $\gamma_1 CF_{1(ij)} + \dots + \gamma_k CF_{k(ij)}$ corrects for observed confounder effects, with CF_1, \dots, CF_k a set of k confounders. To obtain an analysis comparing cases with controls within pairs, we need to adjust for between-pairs heterogeneity, through assumption of the random effect $\alpha_i \approx N(0, \sigma_\alpha^2)$. Conditional on both the between-pairs random effect and observed confounder adjustments, outcomes are assumed exchangeable with this model through assumption of residual normal error. We refer to Verbeke and Molenberghs (2001) for further details on the model.

For the cortisol data the mixed models were corrected for month of measurements (dark months: October through February; light months: March through September), time of awakening, and smoking (number of cigarettes a day), as was derived from yet unpublished data of our own center on determinants of salivary cortisol in 494 healthy controls. For the sAA data the mixed models were corrected for the use of coffee, smoking and the Body Mass Index as was based on the review of Granger et al. (2007). The evaluation of cortisol levels in the evening and sAA and cortisol levels subsequent to dexamethasone intake was based on separate analyses of the measurements taken at test day I at 2200 h and at test day II at 900 h and 1600 h, using the above described mixed model approach. For this statistical model, the assumption is that the residuals are normally distributed after the incorporation of the confounding factors in the model. Here, the residuals of both the sAA and the cortisol data were skewed. Therefore we performed a square root transformation for the sAA data, and for cortisol a log transformation, after which the residuals were investigated anew and found to be of normal distribution.

2. Separate analyses were implemented for patients and controls to investigate the presence of a cortisol awakening rise (CAR). Paired t -tests (two-sided) were carried out for cortisol measurements at 30 minutes post-awakening relative to the awakening measurements. These data were normally distributed.

3. All mixed model analyses were carried out through the SPSS 14.0 mixed model routine.

We are purposely presenting both the raw, unadjusted p -values as reported by the analyses and the Bonferroni corrected p -values.

Results

Subjects

Initially 46 patients and 54 controls were screened for this study. Three patients and eleven controls dropped out. For more detailed information on this see the flow chart in Figure 1. Forty-three patients with gSAD and 43 age (± 5 years) and gender matched healthy controls participated in this study. Subject characteristics are given in Table 1. The gSAD group was assessed within 13 months, the control subjects within 2.5 years.

Of the 43 patients and controls, some data were missing due to insufficient saliva collection. For all missing data, the matching data from the other group were removed from the analyses as well. Insufficient saliva was collected for cortisol measurement of test day II at 900 h in three subjects ($n=40$), and of cortisol test day II at 1600 h in one subject ($n=42$). Insufficient saliva was collected for sAA measurements of the AUC awakening in one subject ($n=42$) and at test day II 900 h in one subject ($n=42$). After the test days it appeared that one patient used a β -blocker for high blood pressure, but did not mention this before. The data of this patient were included in the analyses.

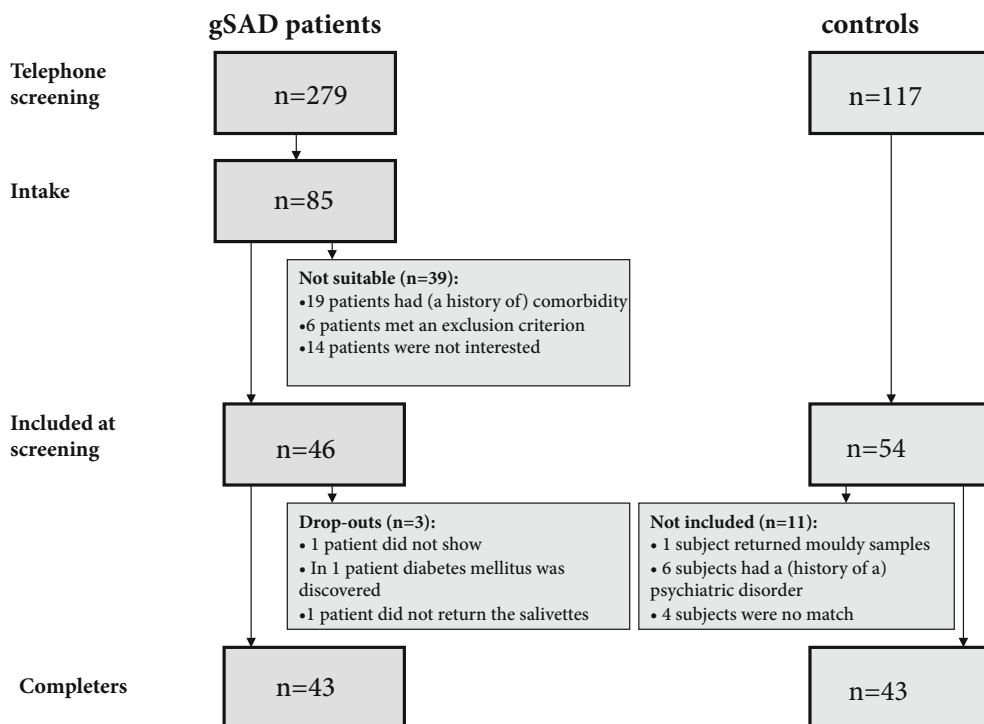


Figure 1. Flow chart of recruitment and inclusion process of gSAD patients and controls. The numbers of patients and controls that entered the various stages of the recruitment and inclusion process.

Table 1 Characteristics of gSAD patients and controls.

	gSAD patients (n=43)	Healthy controls (n=43)
CHARACTERISTICS		
Number of women / men	21 / 22	21 / 22
Age women / men (years)	42.1 (\pm 11.8) / 41.3 (\pm 13.9)	41.7 (\pm 12.2) / 41.4 (\pm 14.5)
Age total (years)	41.7 (\pm 12.8)	41.6 (\pm 13.3)
Smoking	16.3% (n=7)	14.0% (n=6)
Number cigarettes/day	8.6 (\pm 6.3)	10.3 (\pm 5.7)
Use of alcohol	86.0% (n=37)	74.4% (n=32)
Number of units alcohol/week	6.6 (\pm 4.2)	5.7 (\pm 3.7)
BMI	23.9 (\pm 3.4)	24.4 (\pm 3.9)
LSAS score	78.2 (\pm 19.6)	8.6 (\pm 5.6)
HRDS score	5.7 (\pm 3.1)	1.3 (\pm 1.5)
Trauma	49% (n=21)	40% (n=17)
MEDICATION		
Beta-blockers	2% (n=1)	0%
Oxazepam 10 mg (as needed)	2% (n=1)	2% (n=1)
Nasal glucocorticoids	2% (n=1)	0%
Inhaled glucocorticoids	2% (n=1)	2% (n=1)
Glucocorticoid crème	0%	5% (n=2)
Oral contraceptives	19% of women (n=4)	38% of women (n=8)

gSAD=generalized social anxiety disorder; BMI=body mass index; LSAS=Liebowitz Social Anxiety Scale; HRDS=Hamilton Rating Scale for Depression

Salivary cortisol

At test day I, we found no differences between gSAD patients and controls on the AUC cortisol after awakening as analyzed with mixed models and corrected for the confounders ($p=0.864$) (see Figure 2 and Table 2). In each group we compared cortisol levels at 30 minutes after awakening with cortisol levels directly at awakening with a paired sample t-test. In both the gSAD and control group no significant differences were found between these time points (mean = -2.62, S.D. = 9.23, $p = 0.07$; mean = -0.035, S.D. = 5.56, $p = 0.967$), reflecting the absence of a CAR in both groups (see Figure 2).

Mixed models analysis with correction for confounders also showed no differences between both groups in the AUC diurnal cortisol and the cortisol level at 2200 h ($p=0.773$; $p=0.201$) (see Figure 2 and Table 2).

Compliance was checked in both groups. Dexamethasone was present in the saliva at day II of all subjects, except for three controls and one patient. Since an obvious suppression of their cortisol levels was found, they probably did ingest the dexamethasone. At test day II post-dexamethasone cortisol levels (900 h and 1600 h) did not differentiate between the groups ($p=0.920$; $p=0.256$) (see Table 2 and Figure 2).

Taken together, we did not observe significant differences in saliva cortisol at any measure of the HPA-axis we used.

Salivary alpha-amylase

In contrast, sAA did show statistically significant differences between patients and controls as analyzed with mixed models, after correction for the confounding factors. On test day I in the morning, during the first hour after awakening, we did not find significant differences in the AUC sAA awakening ($p=0.114$) (see Figure 3 and Table 2). However, during the day (from 1100 h to 2200 h), the AUC sAA diurnal was much higher in gSAD patients as compared to controls ($p=0.022$). At 1500 h sAA levels were almost twice as high in patients as compared to controls. For details see Figure 3 and Table 2.

On test day II, post-dexamethasone sAA levels were significantly higher in gSAD patients compared to controls at 1600 h ($p=0.02$) (see Table 2, and Figure 3), but not at 900 h ($p=0.059$) (see Table 2 and Figure 3). In Table 2 also the Bonferroni corrected p -values are given.

Table 2 Cortisol (log transformed) and sAA (sqrt transformed) data in gSAD patients and controls as analyzed with mixed models (within matched pairs)

		Mean gSAD	Co	<i>F</i>	<i>p</i>	<i>p</i> (bonf. corr.)	<i>n</i>
Cortisol (nmol/l)							
Day I	AUC awakening	962.2	1060.9	0.030	0.864	-	43
	AUC diurnal	4993.6	5486.5	0.084	0.773	-	43
	Late evening (2200 h)	3.5	4.5	1.687	0.201	-	43
Day II	Post-dex. 900 h	4.1	4.5	0.010	0.920	-	40
	Post-dex. 1600 h	3.384	3.029	1.307	0.256	-	42
Amylase (U/ml)							
Day I	AUC awakening	9299.145	6959.123	2.612	0.114	-	42
	AUC diurnal	225147.69	146808.58	5.413	0.022*	0.044*	43
Day II	Post-dex. 900 h	191.776	130.758	3.794	0.059	-	42
	Post-dex. 1600 h	327.542	206.047	5.628	0.020*	0.040*	43

sAA=salivary alpha-amylase; gSAD=generalized social anxiety disorder; Co=controls; AUC awakening = the area under the curve of cortisol levels at awakening, 30 minutes, 45 minutes and 60 minutes after awakening; AUC diurnal= the area under the curve of cortisol levels at 1100 h, 1500 h, 1900 h and 2200 h. Late evening cortisol is the cortisol level at 2200 h. Post-dex. are the data following the ingestion of 0.5 mg dexamethasone. These data reflect mixed models analysis of the cortisol and sAA data, corrected for confounders. The cortisol data were log-transformed. For the sAA data a square root transformation was performed. The F and p -values reflect analyses with the transformed data. P (bonf. corr.) reflects the bonferroni corrected significant p -values. * means that the data were statistically significant. For cortisol the confounders that were taken into account were month of measurements, time of awakening, and smoking, for sAA the confounders that were taken into account were use of coffee, smoking, and the Body Mass Index.

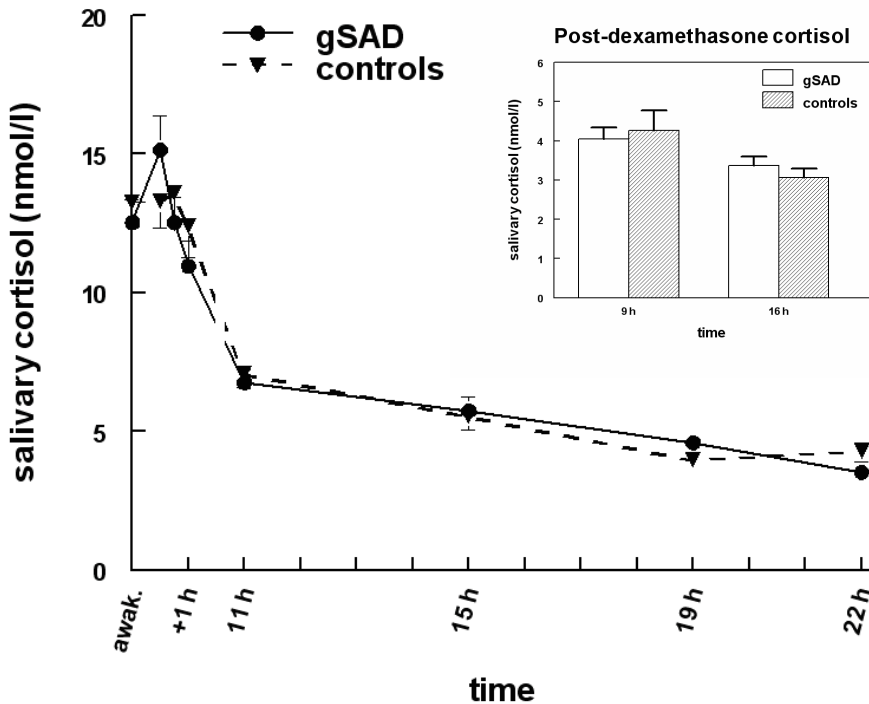


Figure 2. Awakening, diurnal and post-dexamethasone salivary cortisol levels (\pm S.E.M.) in gSAD patients and controls. Awak. = awakening samples, they were taken at the moment of awakening, and after 30 minutes, 45 minutes and 1 hour after awakening at test day I. The diurnal samples were taken at 1100 h, 1500 h, 1900 h and 2200 h at test day I. Post-dexamethasone salivary cortisol levels: 900 h and 1600 h are the time points at test day II, the day following the ingestion of dexamethasone.

Discussion

Here we show that gSAD patients differ from healthy controls in their diurnal sAA and post-dexamethasone sAA level at 1600 h, but not in their cortisol levels. This suggests an imbalance between the two branches of the stress system. Several arguments indicate that the increased diurnal sAA levels really indicate higher ANS activity in gSAD. However, as discussed in the introduction, previous studies in gSAD with traditional markers of ANS activity like (nor)adrenaline, heart rate and blood pressure were contradictory. First this could be explained by the findings that sAA as a marker for the ANS is relatively independent from several possible confounding factors as was reported before (Nater et al., 2007), while (nor)adrenaline levels, heart rate and blood pressure, are easily influenced by many factors, including posture and venipuncture. Second, we assessed sAA several times a day contrary to the single or closely clustered assessments of the 'classical' parameters.

The diurnal sAA levels and patterns we describe here were very similar to the sAA curves that were found before (Rohleder et al., 2004; Nater et al., 2007), indicating the validity of our results. Although it was not the aim of our study, we found no effects of dexamethasone on sAA. This means that the post-dexamethasone ANS hyperactivity we found in gSAD probably also reflects basal hyperactivity as we found on test day I.

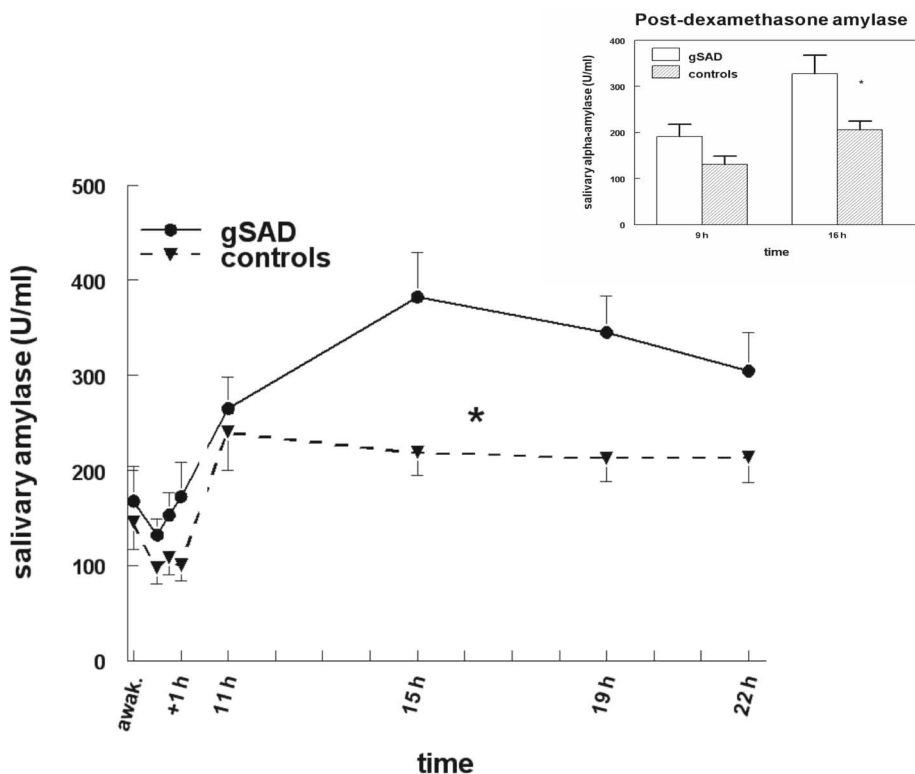


Figure 3. Awakening, diurnal and post-dexamethasone salivary alpha-amylase levels (\pm S.E.M.) in gSAD patients and controls. Awak. = awakening samples, they were taken at the moment of awakening, and after 30 minutes, 45 minutes and 1 hour after awakening at test day I. The diurnal samples were taken at 1100 h, 1500 h, 1900 h and 2200 h at test day I. Post-dexamethasone salivary alpha-amylase levels: 900 h and 1600 h are the time points at test day II, the day following the ingestion of dexamethasone.

* means a statistical difference between patients and controls in sAA levels as analyzed with mixed models ($p=0.009$).

With respect to cortisol levels we did not find differences between gSAD patients and healthy controls, as was previously shown by others (Potts et al., 1991; Uhde et al., 1994; Martel et al., 1999).

In contrast to what might be expected, neither in the gSAD patients nor in the controls a cortisol awakening rise (CAR) was found. This may be the consequence of asking the participants to sample saliva cortisol during rest days. It has been reported that the CAR is smaller in weekend days as opposed to working days (Kunz-Ebrecht et al., 2004). It also might be the effect of non-adherence to the protocol (Thorn et al., 2006), the effect of the time of awakening, which apparently is different in weekend days (Clow et al., 2004), or the effect of the great variation of cortisol levels in the general population.

No statistically significant differences between the groups were observed in the DST, with both groups showing suppression of cortisol. HPA-axis suppression in the gSAD group was comparable to those in healthy controls being in line with a previous report (Uhde et al., 1994). Thus it could be concluded from the present study that in basal conditions the HPA-axis function is

normal, whereas there is an increased activity of the autonomic system itself.

We were the first to investigate the HPA-axis and the ANS together in basal non-stimulated conditions in gSAD, and had the opportunity to directly compare these two systems in the same group of patients and controls. Other strengths of the study are that we used a relatively large group of patients with individually matched controls. The patients were suffering from pure gSAD, no life-time comorbidity was allowed. For cortisol we tried to prevent the influence of confounding factors in several ways. Patients and controls were matched for age and gender. Subjects that were heavy smokers or drinkers were excluded. Furthermore, we corrected for several remaining confounding factors in the statistical analyses. For sAA we also took several possible confounders into account, based on the review of Granger et al. (Granger et al., 2007). Another strength is that cortisol and sAA were measured in saliva (Gozansky et al., 2005; Rohleder et al., 2006), which has the advantage of non-stressful sampling as opposed to measurements in blood. In addition, saliva samples were collected at home, on non-working and non-stressful days, without engaging in social situations. This approach secures equal conditions in both groups, without confounding factors as anticipatory and social anxiety in the gSAD group.

In asking participants to sample saliva at home we optimized the chances that assessments took place under basal conditions. In doing so we followed many other studies (Clow et al., 2004). However, unsupervised sampling at home may decrease compliance. We took several measures to control for this possibility. First, we stimulated compliance by asking the participants to write down the time of sampling and the activities they did the hour before sampling, and by informing them about our plans to check compliance, as it has been demonstrated that such a procedure enhances compliance (Kudielka et al., 2003). Second, we checked saliva dexamethasone levels and found no indications of non-compliance in any of the participants. Nevertheless, we cannot rule out that non-compliance to the protocol influenced our data on cortisol as well as sAA. Hence, we might have missed existing differences between gSAD patients and controls at the cortisol and sAA awakening responses. Furthermore, our data might have been of more value if we had measured full awakening responses of both markers after the DST.

In conclusion, under basal conditions we found no changes in HPA-axis activity but, in contrast, an increased autonomic activity. This suggests an imbalance in the stress-system probably by relative increased activity of the autonomic system as compared to the HPA-axis. These findings are in line with the hyperarousal observed in gSAD patients, as it might perhaps be the result of the hyperactivity of the ANS. Alternatively, the hyperactivity of the ANS might be the result of the chronic stress accompanying the continuing fear to be humiliated by others. Further research could be directed at the associations between hyperarousal and sAA in gSAD and at differences in ANS activity between gSAD and other anxiety disorders.

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References

- American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders DSM-IV Text Revision* American Psychiatric Press, Washington, DC.
- Bosch, J. A., Brand, H. S., Ligtenberg, T. J., Bermond, B., Hoogstraten, J., and Nieuw Amerongen, A. V. (1996). Psychological stress as a determinant of protein levels and salivary-induced aggregation of *Streptococcus gordonii* in human whole saliva. *Psychosom.Med.*, 58, 374-382.
- Bouwer, C. and Stein, D. J. (1998). Hyperresponsivity to nicotinic acid challenge in generalized social phobia: a pilot study. *Eur.Neuropsychopharmacol.*, 8, 311-313.
- Chatterton, R. T., Jr., Vogel song, K. M., Lu, Y. C., Ellman, A. B., and Hudgens, G. A. (1996). Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin.Physiol*, 16, 433-448.
- Chatterton, R. T., Jr., Vogel song, K. M., Lu, Y. C., and Hudgens, G. A. (1997). Hormonal responses to psychological stress in men preparing for skydiving. *J.Clin.Endocrinol.Metab*, 82, 2503-2509.
- Clow, A., Thorn, L., Evans, P., and Hucklebridge, F. (2004). The awakening cortisol response: methodological issues and significance. *Stress.*, 7, 29-37.
- Condren, R. M., O'Neill, A., Ryan, M. C., Barrett, P., and Thakore, J. H. (2002). HPA axis response to a psychological stressor in generalised social phobia. *Psychoneuroendocrinology*, 27, 693-703.
- Draijer, N. (1989). Structured Trauma Interview. Department of Psychiatry, Free University, Amsterdam.
- De Kloet, E. R., Joels, M., and Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat.Rev. Neurosci.*, 6, 463-475.
- Ehlert, U., Erni, K., Hebis ch, G., and Nater, U. (2006). Salivary alpha-amylase levels after yohimbine challenge in healthy men. *J.Clin.Endocrinol.Metab*, 91, 5130-5133.
- Furlan, P. M., DeMartinis, N., Schweizer, E., Rickels, K., and Lucki, I. (2001). Abnormal salivary cortisol levels in social phobic patients in response to acute psychological but not physical stress. *Biol.Psychiatry*, 50, 254-259.
- Gaab, J., Huster, D., Peisen, R., Engert, V., Schad, T., Schurmeyer, T. H. et al. (2002). Low-dose dexamethasone suppression test in chronic fatigue syndrome and health. *Psychosom.Med.*, 64(2), 311-318.
- Gerlach, A. L., Wilhelm, F. H., and Roth, W. T. (2003). Embarrassment and social phobia: the role of parasympathetic activation. *J.Anxiety Disord.*, 17, 197-210.
- Gilman, S. C., Fischer, G. J., Biersner, R. J., Thornton, R. D., and Miller, D. A. (1979). Human parotid alpha-amylase secretion as a function of chronic hyperbaric exposure. *Undersea Biomed.Res.*, 6, 303-307.
- Goldstein, D. S. (2003). Catecholamines and stress. *Endocr.Regul.*, 37, 69-80.
- Gozansky, W. S., Lynn, J. S., Laudenslager, M. L., and Kohrt, W. M. (2005). Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic-pituitary-adrenal axis activity. *Clin.Endocrinol.(Oxf)*, 63, 336-341.
- Granger, D. A., Kivlighan, K. T., el Sheikh, M., Gordis, E. B., and Stroud, L. R. (2007). Salivary alpha-amylase in biobehavioral research: recent developments and applications. *Ann.N.Y.Acad.Sci.*, 1098, 122-144.
- Grossman, P., Wilhelm, F. H., Kawachi, I., and Sparrow, D. (2001). Gender differences in psychophysiological responses to speech stress among older social phobics:: congruence and incongruence between self-evaluative and cardiovascular reactions. *Psychosom.Med.*, 63, 765-777.
- Hamilton, M. (1960). A rating scale for depression. *J.Neurol.Neurosurg.Psychiatry*, 23, 56-62.
- Kivlighan, K. T. and Granger, D. A. (2006). Salivary alpha-amylase response to competition: relation to gender, previous experience, and attitudes. *Psychoneuroendocrinology*, 31, 703-714.
- Kudielka, B. M., Broderick, J. E., and Kirschbaum, C. (2003). Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosom.Med.*, 65, 313-319.
- Kunz-Ebrecht, S. R., Kirschbaum, C., Marmot, M., and Steptoe, A. (2004). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology*, 29, 516-528.
- Laederach-Hofmann, K., Mussgay, L., Buchel, B., Widler, P., and Ruddel, H. (2002). Patients with erythropho-

- bia (fear of blushing) show abnormal autonomic regulation in mental stress conditions. *Psychosom.Med.*, 64, 358-365.
- Liebowitz, M. R. (1987). Social phobia. *Mod.Probl.Pharmacopsychiatry*, 22:141-73., 141-173.
- Lorentz, K. (1998). Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 9. IFCC method for alpha-amylase (1,4-alpha-D-glucan 4-glucanohydrolase, EC 3.2.1.1). International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Committee on Enzymes. *Clin.Chem.Lab Med.*, 36, 185-203.
- Martel, F. L., Hayward, C., Lyons, D. M., Sanborn, K., Varady, S., and Schatzberg, A. F. (1999). Salivary cortisol levels in socially phobic adolescent girls. *Depress.Anxiety*, 10, 25-27.
- Nater, U. M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M. M. et al. (2006). Stress-induced changes in human salivary alpha-amylase activity - associations with adrenergic activity. *Psychoneuroendocrinology*, 31, 49-58.
- Nater, U. M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C. et al. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int.J.Psychophysiol.*, 55, 333-342.
- Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., and Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, 32, 392-401.
- Potts, N. L., Davidson, J. R., Krishnan, K. R., Doraiswamy, P. M., and Ritchie, J. C. (1991). Levels of urinary free cortisol in social phobia. *J.Clin.Psychiatry*, 52 Suppl:41-2., 41-42.
- Rohleder, N., Nater, U. M., Wolf, J. M., Ehlert, U., and Kirschbaum, C. (2004). Psychosocial stress-induced activation of salivary alpha-amylase: an indicator of sympathetic activity? *Ann.N.Y.Acad.Sci.*, 1032, 258-263.
- Rohleder, N., Wolf, J. M., Maldonado, E. F., and Kirschbaum, C. (2006). The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. *Psychophysiology*, 43, 645-652.
- Stein, M. B. (2006). An epidemiologic perspective on social anxiety disorder. *J.Clin.Psychiatry*, 67 Suppl 12, 3-8.
- Stein, M. B., Asmundson, G. J., and Chartier, M. (1994). Autonomic responsivity in generalized social phobia. *J.Affect.Disord.*, 31, 211-221.
- Stein, M. B., Tancer, M. E., and Uhde, T. W. (1992). Heart rate and plasma norepinephrine responsivity to orthostatic challenge in anxiety disorders. Comparison of patients with panic disorder and social phobia and normal control subjects. *Arch.Gen.Psychiatry*, 49, 311-317.
- Thorn, L., Hucklebridge, F., Evans, P., and Clow, A. (2006). Suspected non-adherence and weekend versus week day differences in the awakening cortisol response. *Psychoneuroendocrinology*, 31, 1009-1018.
- Uhde, T. W., Tancer, M. E., Gelernter, C. S., and Vittone, B. J. (1994). Normal urinary free cortisol and post-dexamethasone cortisol in social phobia: comparison to normal volunteers. *J.Affect.Disord.*, 30, 155-161.
- Van Aken, M.O., Romijn, J.A., Miltenburg, J.A., Lentjes, E.G. (2003). Automated measurement of salivary cortisol. *Clin. Chem.*, 49, 1408-1409.
- Van Stegeren, A., Rohleder, N., Everaerd, W., and Wolf, O. T. (2006). Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology*, 31, 137-141.
- Van Vliet, I. M. and De Beurs, E. (2007). The MINI-International Neuropsychiatric Interview. A brief structured diagnostic psychiatric interview for DSM-IV en ICD-10 psychiatric disorders. *Tijdschr.Psychiatr.*, 49, 393-397.
- Verbeke, G. and Molenberghs, G. (2001). Linear Mixed Models for Longitudinal Data. Springer-Verlag.
- Weijtens, O., van der Sluijs, F. A., Schoemaker, R. C., Lentjes, E. G., Cohen, A. F., Romijn, F. P. et al. (1997). Peribulbar corticosteroid injection: vitreal and serum concentrations after dexamethasone disodium phosphate injection. *Am.J.Ophthalmol.*, 123, 358-363.

Tryptophan depletion affects the autonomic stress response in generalized social anxiety disorder

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Abstract

In generalized social anxiety disorder (gSAD), serotonergic dysfunctions are found, as well as abnormalities of the autonomic nervous system (ANS) in basal conditions and of the hypothalamic pituitary adrenal (HPA) axis in response to psychological challenges. These findings raise the question whether these phenomena are interrelated. Therefore we designed a study in which two groups with nine pair wise age and gender matched gSAD patients (total of 10 men and 8 women), who were successfully treated with a selective serotonin reuptake inhibitor (SSRI), 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 the promising new marker salivary alpha-amylase (sAA), and HPA-axis responses, as measured with salivary cortisol. The most important result was that the TD group showed a significant larger sAA response to the public speaking task 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 more than the HPA-axis.

Introduction

Several biological systems behave differently in generalized social anxiety disorder (gSAD). With respect to the serotonergic system higher binding potentials for the serotonin transporter in the thalamus and reduced 5-HT_{1A} receptors levels have been found (see among others (Van Der Wee et al., 2008). In addition, challenges with various serotonergic agonists and tryptophan depletion induced exaggerated responses (see among others (Van Veen et al., 2007). Besides, the favorable effects of selective serotonin reuptake inhibitors (SSRIs) in gSAD suggest serotonergic involvement (Blanco et al., 2003). A reciprocal interaction between the central serotonergic and noradrenergic systems has been proposed (Tassin, 2008), suggesting that SSRIs could also modulate autonomic function.

The autonomic nervous system (ANS) and the Hypothalamic Pituitary Adrenal-axis (HPA-axis) behave also different in gSAD. In basal, non-stressed conditions, diurnal hyperactivation of the ANS was found, as measured with the promising new marker salivary alpha-amylase (sAA) (Van Veen et al., 2008). During psychological stress the increase in systolic blood pressure and heart rate was larger than in normal controls (see among others Gerlach et al., 2003). With respect to the HPA-axis in basal conditions no change was found (Van Veen et al., 2008), but psychological stress induced hyperfunction (see among others (Condren et al., 2002).

Taken together, this research leads to the question whether the serotonergic system, ANS and HPA-axis are interrelated in gSAD, and, more specifically, whether manipulation of the serotonergic system with, for example, SSRIs leads to alterations in ANS and HPA-axis function.

In this paper we report about the effects of a tryptophan depletion (TD) challenge compared to placebo on ANS and HPA-axis responses to public speaking stress in SSRI-treated gSAD patients. Acute TD is a procedure that temporarily decreases serotonergic neurotransmission, decreasing the efficacy of SSRIs (Hood et al., 2005). The stress system was activated by means of a public speaking challenge. Anticipatory anxiety and learning were avoided by dividing the gSAD patients in two groups, instead of using a crossover design. The neuroendocrine parameters sAA, as a marker of the ANS, and cortisol, as a marker of the HPA-axis, were measured. Based on our findings in basal conditions (Van Veen et al., 2008) we expected to find the ANS to be more sensitive to stress than the HPA-axis.

Methods

Subjects

Eighteen patients with gSAD (10 men, 8 women), pair wise matched on age and gender, were randomly assigned to two conditions, TD and placebo. They were responders to 20 weeks of treatment with citalopram 20-60 mg a day. No life-time psychiatric comorbidity (confirmed with the MINI Plus 5.0.0) (see also Van Veen et al., 2008) or clinically significant medical disorders, such

as endocrinological disorders, were allowed. Before the test day the Liebowitz Social Anxiety Scale (see also Van Veen et al., 2008) and the Beck Depression Inventory (BDI) were used to measure symptom severity (Beck and Steer, 1987).

In case of heavy smoking, abuse of alcohol, or use of drugs of abuse subjects were excluded. Use of psychotropic medication (including beta-blocking agents) had to be stopped at least 14 days before the trial. Women were tested during the follicular phase of the menstrual cycle and women using oral contraceptives in the stop week. Perimenopausal women were excluded, postmenopausal women were included.

Procedures

The protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center. The study was carried out in accordance with the Declaration of Helsinki. All procedures were conducted with the adequate understanding and written informed consent of the subjects.

At the test day an indwelling catheter was placed in an antecubital vein. At 1000h the TD amino-acid mixture or placebo was ingested. At 1525h patients received instructions for the public speaking task. At 1600h the public speaking task started, and ended at 1610h. The test day ended at 1700h.

For the TD patients fasted overnight and were kept on a low protein diet during the test day until the following morning. The TD amino-acid drinks we used were the standard 100 mg drink (see Hood et al., 2005). During the test day, LNAAs, total TRP and 5-hydroxy-tryptophan (5-HTP) were assessed. The public speaking test was based on the principles of the Trier Social Stress Test (Kirschbaum et al., 1993), but slightly modified. Patients were given 15 minutes to prepare a 10 minute speech on a subject of choice. It was suggested (but not the case) that they were judged by an audience behind the one-way mirror-wall and that the whole session would be video-taped.

During the test day, plasma was obtained, behavioural measurements such as the Visual Analogue Scale (VAS) Anxiety and a short version of the Profile of Mood States (POMS) (Wald and Mellenbergh, 1990), and physiological assessments, such as heart rate and tension (Dinamap[®] Pro 100), were done at baseline (t0), after the TD (t1), after the preparation for the public speaking challenge (t2), and after the public speaking challenge (t3). Saliva for cortisol and sAA measurements was first collected after the TD and thereafter at 12 time points until 7 hours after the public speaking challenge.

Neuroendocrine assessments

TRP, TRP/LNAA and 5-HTP

Plasma total TRP, the LNAAs phenylalanine, tyrosine, TRP, isoleucine, leucine and valine, and 5-HTP were assessed to evaluate the efficacy of the TD procedure. For the amino acids, quantitative amino-acid analysis was performed by high-performance liquid chromatography as described elsewhere (Fekkes et al., 1995). The ratio total TRP / LNAA was calculated as 100 times

the concentration of TRP divided by the summed concentrations of the other LNAAs. For the 5-HTP assay, see (Gijsman et al., 2002). The lower limits of detection and quantification were 0.5 and 1.7 ng/ml, respectively. The coefficients of variability for precision and reliability were 2.6% and 7.9%, respectively (Gijsman et al., 2002).

sAA and cortisol

The determination of sAA and cortisol was described in our previous study (Van Veen et al., 2008).

Statistics

Since the subjects were pair wise matched, the TD and placebo group were compared with paired-samples *t*-tests.

1. For baseline characteristics, the groups were compared on age, severity of gSAD symptoms after treatment (LSAS score), depressive symptomatology (BDI score), smoking, the use of alcohol and dosage of citalopram.
2. The effects of the TD alone (after the TD and before the public speaking challenge) on sAA_{t1}, cortisol_{t1}, VAS anxiety_{Δt1-t0}, POMS_{Δt1-t0}, heart rate_{Δt1-t0}, and systolic_{Δt1-t0} and diastolic_{Δt1-t0} blood pressure were analyzed.
3. The neuroendocrine data in response to the public speaking test were normalized by dividing all sAA and cortisol values by the first value (t1), which was obtained before the public speaking challenge. The normalized data show the relative increase in sAA and cortisol in response to the public speaking challenge. We normalized the data because of the small sample sizes, large variations between subjects in individual sAA levels, and to take into account the possible influences of the TD/placebo condition on the neuroendocrine data before the start of the public speaking challenge.
4. The TD and placebo group were compared on relative sAA and cortisol levels after the public speaking challenge (t3). Also the VAS anxiety_{Δt3-t1}, POMS_{Δt3-t1}, heart rate_{Δt3-t1}, systolic_{Δt3-t1} and diastolic_{Δt3-t1} blood pressure at this time point were analyzed.

All analyses were done with SPSS 16.0.

Results

Subjects

The TD and placebo group showed no significant differences on age ($p=0.74$; mean of both groups together 39.1), symptom severity ($p=0.15$; mean LSAS score 28.6), use of alcohol ($p=0.67$; mean 4 units a week), smoking ($p=0.2$; mean 1.1 cigarette a day) and the dosage of citalopram ($p=0.35$; mean 42.4 mg a day). One patient stopped just after the explanation of the public speaking task, because of acute panic. The data that were already collected were included in the analyses. Unfortunately, it appeared that one patient used sertraline (dosage 100 mg a day) instead of citalopram. This patient

was included in the analyses.

Tryptophan depletion

The TD procedure resulted in a reduction at t1 of total TRP of 90.6%, of the TRP/LNAA ratio of 96.6%, and of 5-HTP of 80.2%. The placebo drink resulted in an increase in total TRP of 84.3%, a decrease of the TRP/LNAA ratio of 46.3%, and an increase of 5-HTP of 12.6%.

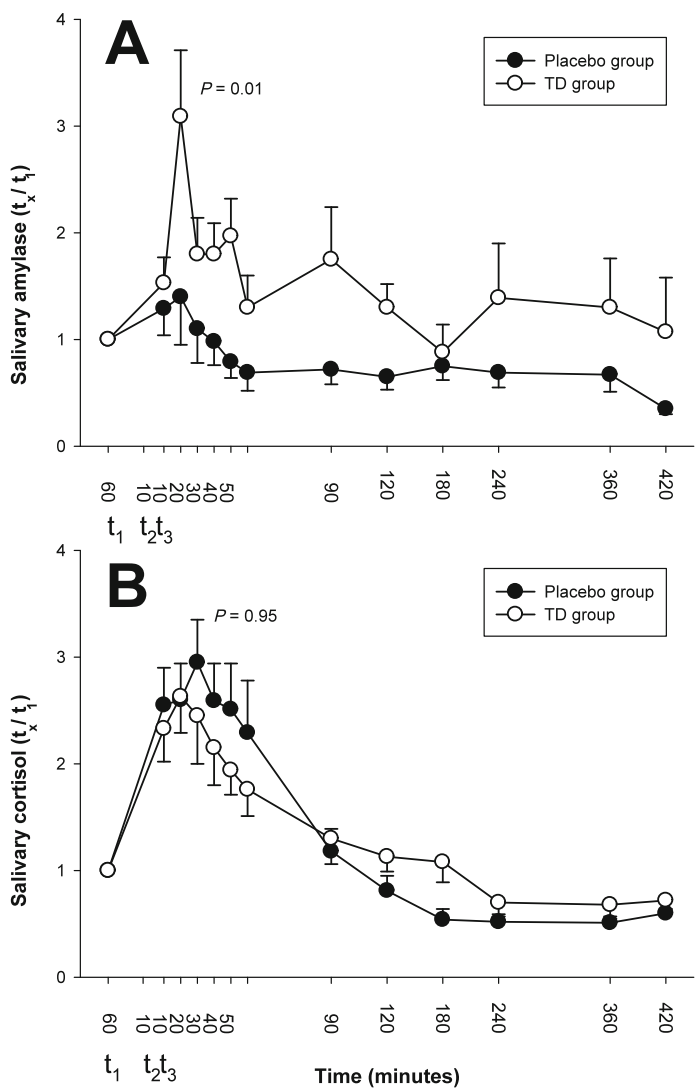


Figure 1. The relative (normalized data) sAA and cortisol responses (\pm S.E.M.) before and after the public speaking challenge. The p-value is the result of the comparison of the relative increases at t3 between the TD and placebo group by means of a paired-samples *t*-test: t1 is after the TD challenge and before the public speaking challenge; t2 is after the preparation phase and before the public speaking challenge; t3 is after the public speaking challenge.

Table 1 Relative increases of sAA and cortisol (t3) and Δ_{t3-t1} mood and anxiety scores in response to a TD directly after a public speaking challenge.

	<i>t</i> -Value	Mean difference (TD – placebo)	95% CI (lower-upper)	<i>p</i> -value
sAA _{t3}	-3.29	-1.69	-2.91 to -0.48	0.013*
cortisol _{t3}	-0.06	-0.03	-1.28 to 1.22	0.95
Δ_{t3-t1} VAS-anxiety	-0.78	-7.75	-31.35 to 15.85	0.46
Δ_{t3-t1} POMS	0.16	-0.63	-8.89 to 10.14	0.88
Δ_{t3-t1} heart rate	-0.77	-5.88	-24.03 to 12.28	0.47
Δ_{t3-t1} systolic BP	-0.54	-4.75	-25.43 to 15.93	0.60
Δ_{t3-t1} diastolic BP	-2.04	-6.00	-12.95 to 0.95	0.08

Paired-samples *t*-test analyses; for both groups *n*=8. * means that the *p*-value is statistically significant. sAA = salivary alpha-amylase; TD = tryptophan depletion; VAS-anxiety = anxiety levels measured with a visual analogue scale; POMS = total scores of the Profile of Mood States; BP = blood pressure; t1 = 1500h, just before the preparation phase; t3 = 1610h, just after the public speaking challenge.

Neuroendocrine assessments

The effects of the TD/placebo condition alone on sAA and cortisol (t1) revealed no statistical differences (*p* = 0.124, *p* = 0.394). However, significant increases in the relative sAA responses to the public speaking task (t3) were seen in the TD group compared to placebo (*p*=0.013; bonferroni corrected *p*-value = 0.026). No significant differences were seen in relative cortisol increases (*p*=0.95). For details see Figure 1 and Table 1.

Behavioural and physiological assessments

No statistical significant differences were found in heart rate, blood pressure, and anxiety and mood assessments between the TD and the placebo condition.

Discussion

The most important finding of this study is that in successfully SSRI-treated gSAD patients sAA responses to a social stress test were significantly higher in the TD condition as compared to placebo. No differences were found in salivary cortisol, as well as anxiety, mood, blood pressure and heart rate responses. The TD condition without public speaking did not differ from the placebo condition in sAA, cortisol, anxiety, depressive symptoms, blood pressure and heart rate.

The higher sAA response to stress in the TD group was not accompanied by higher responses of the more traditional markers of autonomic functioning: heart rate and blood pressure. Although these contradictory results may be due to the small number of subjects, they are also compatible with a modest effect on heart rate and blood pressure. All in all, sAA might be a more sensitive and stable marker for autonomic functioning than heart rate and blood pressure, as it is less influenced by confounding factors like posture and exercise (Nater et al., 2007). The

TD procedure may have decreased the effects of public speaking on sAA concentrations as sAA contains the amino-acid TRP which is depleted. Nevertheless, the TD group showed significantly larger sAA increases as compared to the placebo group.

We did not find an effect of TD on anxiety in response to the public speaking challenge. This finding is in agreement with Argyropoulos et al. (2004). However, Argyropoulos et al. did find effects of TD on anxiety using an autobiographical script. Such a script may be more specific for generalized social anxiety than public speaking is.

To the best of our knowledge, this is the first study to describe the effects of TD on sAA and also the first TD study in gSAD with the assessment of neuroendocrine parameters. The TD procedure induced a profound decrease of TRP and the TRP/LNAA ratio in the TD group. However, in the control group the TRP/LNAA ratio was also decreased with 46.3%, which may be related to the lack of effect seen on the behavioural measures. Strength of the study is that we only included patients with pure gSAD and without medical disorders to rule out the possibility of confounding effects of comorbid disorders on the neuroendocrine data.

In conclusion, the present study shows that in successfully SSRI-treated gSAD patients the stress of public speaking during TD induces higher sAA responses compared to the placebo condition, but has no effect on the HPA-axis. This suggests that in gSAD there is a vulnerability of the ANS more than the HPA-axis, which seems to be mediated by the serotonergic system.

Because of the small number of subjects this study needs replication in a larger group. Besides, future investigations should be directed at the specificity of these findings for gSAD, and of the TD effects in gSAD patients who were treated with cognitive behavioural therapy or noradrenaline reuptake inhibitors. Implications for the future are that, given the involvement of the ANS in gSAD, treatment studies should be directed at the ANS and HPA-axis rather than the HPA-axis alone.

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References

- Argyropoulos, S. V., Hood, S. D., Adrover, M., Bell, C. J., Rich, A. S., Nash, J. R. et al. (2004). Tryptophan depletion reverses the therapeutic effect of selective serotonin reuptake inhibitors in social anxiety disorder. *Biol.Psychiatry*, 56, 503-509.
- Beck, A.T. and Steer, R.A. (1987). Manual for the revised Beck Depression Inventory. San Antonio, TX: Psychological Corporation.
- Blanco, C., Schneider, F. R., Schmidt, A., Blanco-Jerez, C. R., Marshall, R. D., Sanchez-Lacay, A. et al. (2003). Pharmacological treatment of social anxiety disorder: a meta-analysis. *Depress.Anxiety*, 18, 29-40.
- Condren, R. M., O'Neill, A., Ryan, M. C., Barrett, P., and Thakore, J. H. (2002). HPA axis response to a psychological stressor in generalised social phobia. *Psychoneuroendocrinology*, 27, 693-703.
- Fekkes, D., van Dalen, A., Edelman, M., and Voskuilen, A. (1995). Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated pre-column derivatization with o-phthalaldehyde. *J.Chromatogr.B Biomed.Appl.*, 669, 177-186.
- Gerlach, A. L., Wilhelm, F. H., and Roth, W. T. (2003). Embarrassment and social phobia: the role of parasympathetic activation. *J.Anxiety Disord.*, 17, 197-210.
- Gijsman, H. J., van Gerven, J. M., de Kam, M. L., Schoemaker, R. C., Pieters, M. S., Weemaes, M. et al. (2002). Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers. *J.Clin.Psychopharmacol.*, 22, 183-189.
- Hood, S. D., Bell, C. J., and Nutt, D. J. (2005). Acute tryptophan depletion. Part I: rationale and methodology. *Aust.N.Z.J.Psychiatry*, 39, 558-564.
- Kirschbaum, C., Pirke, K. M., and Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, 28, 76-81.
- Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., and Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, 32, 392-401.
- Tassin, J. P. (2008). Uncoupling between noradrenergic and serotonergic neurons as a molecular basis of stable changes in behavior induced by repeated drugs of abuse. *Biochem.Pharmacol.*, 75(1), 85-97.
- Van Der Wee, N. J., Van Veen, J. F., Stevens, H., van Vliet, I. M., van Rijk, P. P., and Westenberg, H. G. (2008). Increased serotonin and dopamine transporter binding in psychotropic medication-naïve patients with generalized social anxiety disorder shown by 123I-beta-(4-iodophenyl)-tropane SPECT. *J.Nucl.Med.*, 49(5), 757-763.
- Van Veen, J. F., Van Der Wee, N. J., Fiselier, J., van Vliet, I. M., and Westenberg, H. G. (2007). Behavioural effects of rapid intravenous administration of meta-chlorophenylpiperazine (m-CPP) in patients with generalized social anxiety disorder, panic disorder and healthy controls. *Eur.Neuropsychopharmacol.*
- Van Veen, J. F., van Vliet, I. M., DeRijk, R. H., van, P. J., Mertens, B., and Zitman, F. G. (2008). Elevated alpha-amylase but not cortisol in generalized social anxiety disorder. *Psychoneuroendocrinology*, 33(10), 1313-1321.
- Wald, F.D.M. and Mellenbergh, G.J. (1990). De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). *Neth. J. of Psychol.*, 45, 86-90.

The effects of female reproductive hormones in generalized social anxiety disorder

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Abstract

Objective: Although generalized social anxiety disorder (gSAD) is more prevalent in women, the role of female reproductive hormones in gSAD has never been investigated. Therefore our aim was to make a first inventory of the influence of female reproductive hormones on gSAD symptoms. **Method:** Female patients with gSAD who had previously participated in our research projects in the University Medical Center Utrecht and the Leiden University Medical Center were recruited. 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. Non-parametric statistical tests were used to analyze the data. **Results:** A subgroup of patients reported an influence of female hormonal cycle on gSAD symptoms. In this subgroup, statistical differences were found for the menstrual cycle and pregnancy. In the premenstrual period, patients reported more severe gSAD symptoms. During pregnancy symptoms decreased, but postpartum symptom severity returned to the same levels as before pregnancy. **Conclusions:** 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.

Introduction

Social Anxiety Disorder (SAD) is one of the most common psychiatric disorders. In a review of European community studies, a lifetime prevalence of 3.9 to 13.7 % for SAD according to DSM-IV criteria was found. Moreover, the prevalence estimates were generally higher in women than in men (Fehm et al., 2005). Two subtypes can be distinguished: specific SAD and generalized SAD (gSAD) (DSM-IV-TR). The onset of gSAD is often in puberty and the symptoms of gSAD might be more severe in women (Keller, 2003; Wittchen and Fehm, 2003). Taken together, these findings might suggest that the gonadal hormones influence the onset and course of gSAD. This hypothesis is supported by literature on gonadal hormones and anxiety. The gonadal hormones estrogen and progesterone regulate the female hormonal cycle: menarche, menstrual cycle, pregnancy, postpartum period, lactation, and menopause. They also affect neurotransmitter systems that are associated with anxiety, such as the dopaminergic, serotonergic, and GABAergic system (for a review: Weinstock, 1999). Furthermore, the female hormonal cycle affects the course and severity of several symptoms of anxiety. Healthy women experienced an increase in anxiety symptoms during the third trimester of pregnancy (Rofe et al., 1993). Premenstrual increase of anxiety symptoms was reported in women with anxiety disorders, such as generalized anxiety disorder (GAD) and panic disorder (PD) (Hsiao et al., 2004; Brambilla et al., 2003). Finally, premenstrual dysphoric disorder and anxiety disorders are highly comorbid conditions (Hsiao et al., 2004).

In gSAD, the influences of female gonadal hormones on anxiety symptoms have never been investigated. The aim of the research described in this article was to explore whether there is an influence of the gonadal hormones on social anxiety and avoidance in women with gSAD. Therefore, we made a retrospective inventory of the course of gSAD symptoms during the female hormonal cycle. Based on the literature in other anxiety disorders, we hypothesized that premenstrually and in the last trimester of the pregnancy the levels of anxiety are higher.

Materials and methods

Participants and procedures

Participants were women over 18 years of age, with gSAD according to DSM-IV criteria, as confirmed with the MINI Plus 5.0.0 (Van Vliet and De Beurs, 2007). Comorbidity, contraception, and hormonal replacement therapy were allowed. Exclusion criterion was an endocrinological disorder of any kind. Patients who did not have a menstruation period for the past year or more were considered postmenopausal.

Female patients with gSAD who had previously participated in our research projects in the University Medical Center Utrecht (UMCU) and the Leiden University Medical Center (LUMC) were recruited.

A total of 140 patients were approached. We sent a letter to 96 UMCU female patients

to ask their permission to call them for participation in the study. We used a directory of old addresses, because these patients did not receive treatment in the UMCU at the time of this study. Reminders were sent out to non-respondents. Patients who returned the permission form were called. We explained the procedures and asked if they wanted to participate. Then the survey was sent. In the LUMC, 44 women who participated in research or were treated for gSAD, were asked to participate during one of their visits. The survey was either given or sent to the patients.

The study was approved by the Medical Ethical Committees of the UMCU and LUMC. All subjects gave written informed consent prior to inclusion in the study.

Survey

Patients received a self-report survey with questions regarding demographic data and the effects of the female hormonal cycle on social anxiety and avoidance. The phases that were studied were menarche, the periods of the menstrual cycle, oral contraceptive (OC) use, pregnancy, lactation, postpartum period, and menopause. For each phase the first question was on the influence of this particular phase on symptoms of social anxiety and of social avoidance. This question could be answered by “yes” or “no”. If the answer was “yes” on this question, they were asked to score the levels of social anxiety and avoidance retrospectively from 0 to 10 for that particular phase. Patients were asked to fill in the questions on the menstrual cycle if they had a regular cycle of 24 to 32 days and only for the time periods in which they had a natural menstrual cycle, without the use of oral contraceptives. Week 1 was considered the menstruation period, week 2 the late follicular phase, week 3 the early luteal phase, and week 4 the premenstrual phase. Questions on pregnancy and postpartum period were directed to the time before pregnancy, the first trimester, the second trimester, the third trimester, and the postpartum period.

Statistics

Descriptive statistics were used for patient characteristics and for some outcome parameters.

Statistical analyses were performed only on data of patients who reported an influence of the female hormonal cycle on symptoms of social anxiety and avoidance, and on current social anxiety and avoidance scores. These were categorical data. We used non-parametric statistics to analyse the data to avoid the normality assumption. Spearman's Rank Order Correlation (ρ) was performed to explore the relationship between the age of menarche and age of onset of gSAD. A Friedman Test was conducted to compare scores on social anxiety and avoidance across different time periods per phase. The Wilcoxon Signed Ranks Test was done for the comparison of social anxiety and avoidance as posthoc analysis for the significant outcome variables of the Friedman Test. This test analyses the direction and the relative magnitude of the differences within pairs.

Results

Response

We sent a letter to 96 female patients with gSAD from the UMCU with information and to ask for permission to call them. Twenty-nine patients (30%) returned the form. They were called and received a survey. Twenty-seven patients (93%) completed the survey. At the LUMC, 44 female patients were asked to participate during a treatment or research visit. All patients volunteered and 37 (84%) returned the survey. Overall, 64 completed surveys (46%) were received.

Patient characteristics

The mean age of the patients was 42.0 years (± 11.7 ; range 21-63 years). The mean age of onset for gSAD was 15.1 years (± 8.4). The delay from the age of onset until the diagnosis gSAD was 22.6 years (± 14.0). Eighty-six percent of the patients received treatment for gSAD during their life, and 45% are currently being treated. A positive family history for gSAD was reported in 50% of the patients. Many patients (75%) were part-time or full-time employed. The remaining 25% were unemployed (14%), incapable of working (3%), retired (3%), or student (5%). Level of education was divided in 3 groups: 24% had a low educational level (10 years or less), 43% had a medium educational level (10-14 years), and 33% had a high educational level (more than 14 years).

The mean current gSAD symptoms on a scale of 0-10 of the entire sample in each area of work, social life, and family life were compared. It appeared that there was a statistically significant difference in social anxiety ($p < 0.001$, $\chi^2 = 73.2$, $df = 2$, $n = 61$) as well as avoidance ($p < 0.001$, $\chi^2 = 62.3$, $df = 2$, $n = 61$) in these three areas. Social anxiety and avoidance are highest in social life and lowest in family life. There was no significant difference in social anxiety and avoidance between work situations and social life ($p = 0.8$; $p = 0.7$), but both were significantly higher than the symptoms in family life ($p < 0.001$ in all cases).

Menarche and age of onset

We found no correlation between age of menarche and age of onset gSAD ($r = -0.01$, $n = 63$, $p = 0.9$).

Influence of female hormonal phase on social anxiety and avoidance

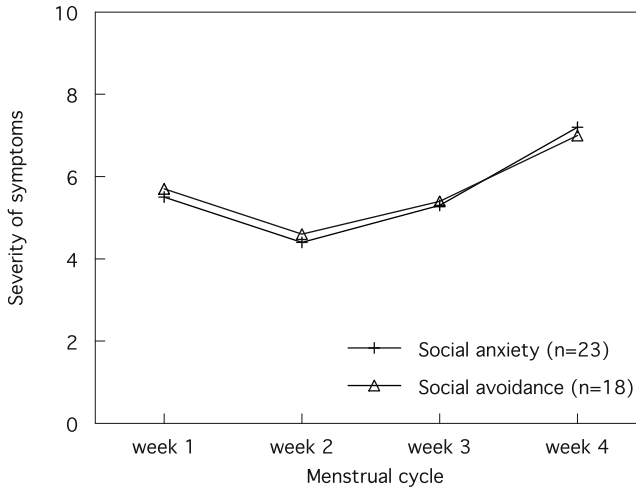
Most patients did not report any influence of menarche, menstrual cycle, OC use, pregnancy, and lactation. However, most patients did notice any influence of the postpartum period and menopause on anxiety symptoms. For exact percentages see Table 1.

Table 1 Influences of female hormonal cycle on gSAD symptoms

Influence on Symptoms	Menarche (n=64)		Menstrual cycle (n= 51)		OC use (n=50)		Pregnancy (n=41)	
	soc.anx.	soc.avoi.	soc.anx.	soc.avoi	soc.anx.	soc.avoi	soc.anx.	soc.avoi.
Yes	13%	8%	45%	35%	12%	14%	41%	41%
No	78%	81%	55%	65%	88%	86%	59%	59%
Unknown	9%	11%						

Influence on Symptoms	Postpartum period (n= 38)		Lactation (n=30)		Menopause (n=13)	
	soc.anx.	soc.avoi	soc.anx.	soc.avoi	soc.anx.	soc.avoi
Yes	56%	53%	3%	7%	69%	62%
No	44%	47%	97%	93%	31%	38%

Note: Percentages of patients who did or did not report an influence on the symptoms of social anxiety (soc.anx.) and avoidance (soc.avoi.).

**Figure 1** The mean social anxiety and avoidance scores (0-10) during the menstrual cycle, as obtained from the patients who reported an influence of the menstrual cycle on social anxiety and avoidance.**Table 2** Statistical differences during the 4 weeks of the menstrual cycle

	Social anxiety (n=23)				Social avoidance (n=18)			
	Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4
Week 1	-	-	-	-	-	-	-	-
Week 2	p=0.02*	-	-	-	p=0.010*	-	-	-
Week 3	p=0.772	p=0.005*	-	-	p=0.532	p=0.016*	-	-
Week 4	p=0.002*	p=0.000*	p=0.000*	-	p=0.015*	p=0.001*	p=0.002*	-

Note: *P*-values of the post hoc analysis with the Wilcoxon Signed Rank Test on the retrospective social anxiety and avoidance scores in the different weeks of the menstrual cycle. Week 1 represents the menstruation, week 2 the late follicular phase, week 3 the early luteal phase, and week 4 the premenstrual phase.

Table 3 Statistical differences before, during, and after the 1st and 2nd pregnancy

Social anxiety 1st pregnancy (n=12)					
	BP	Trim 1	Trim 2	Trim 3	PPP
BP	-	-	-	-	-
Trim 1	p=0.022*	-	-	-	-
Trim 2	p=0.005*	p=0.040*	-	-	-
Trim 3	p=0.003*	p=0.054	p=0.339	-	-
PPP	p=0.301	p=0.016*	p=0.005*	p=0.005*	-
Social avoidance 1st pregnancy (n=10)					
	BP	Trim 1	Trim 2	Trim 3	PPP
BP	-	-	-	-	-
Trim 1	p=0.036*	-	-	-	-
Trim 2	p=0.011*	p=0.071	-	-	-
Trim 3	p=0.007*	p=0.099	p=0.713	-	-
PPP	p=0.889	p=0.167	p=0.090	p=0.056	-
Social anxiety 2nd pregnancy (n=11)					
	BP	Trim 1	Trim 2	Trim 3	PPP
BP	-	-	-	-	-
Trim 1	p=0.070	-	-	-	-
Trim 2	p=0.016*	p=0.014*	-	-	-
Trim 3	p=0.010*	p=0.010*	p=0.102	-	-
PPP		p=0.414	p=0.072	p=0.011*	p=0.007*
Social avoidance 2nd pregnancy (n=9)					
	BP	Trim 1	Trim 2	Trim 3	PPP
BP	-	-	-	-	-
Trim 1	p=0.205	-	-	-	-
Trim 2	p=0.026*	p=0.034*	-	-	-
Trim 3	p=0.017*	p=0.016*	p=0.059	-	-
PPP	p=0.279	p=0.776	p=0.221	p=0.042*	-

Note: *P*-values of the post hoc analysis with the Wilcoxon Signed Rank Test on the retrospective social anxiety and avoidance scores before (BP), and during pregnancy (1st trimester = Trim 1, 2nd trimester = Trim 2, 3rd trimester = Trim 3), and in the postpartum period (PPP) as measured in the first and second pregnancy of the patients who reported an influence of pregnancy and postpartum period on social anxiety and avoidance.

Statistical analyses were performed only on data of patients who reported any influence of the female hormonal cycle on symptoms of social anxiety and avoidance. We report the analyses of the hormonal phases in which more than 10 subjects reported these influences. This was only the case for the menstrual cycle and pregnancy with postpartum period. Seventy-one percent of the patients who reported influences of the menstrual cycle also reported influences of pregnancy when applicable.

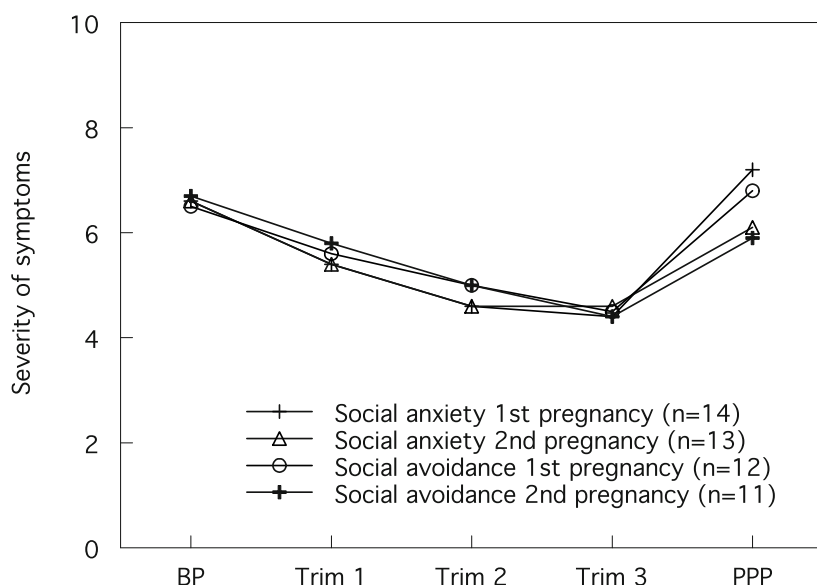


Figure 2. Means of social anxiety and avoidance scores (0-10) before (BP) and during pregnancy (1st trimester = Trim 1, 2nd trimester = Trim 2, 3rd trimester = Trim 3), and in the postpartum period (PPP) as measured in the first and second pregnancy of patients who reported an influence of pregnancy and postpartum period on social anxiety and avoidance.

Menstrual cycle

A difference was found in social anxiety ($p < 0.001$, $\chi^2 = 39.2$, $df = 3$, $n = 23$) and avoidance ($p < 0.001$, $\chi^2 = 26.6$, $df = 3$, $n = 18$) between the 4 weeks of the menstrual cycle. The symptoms of social anxiety and avoidance were highest in week 4 of the menstrual cycle, and significantly different from week 1, 2 and 3. For more details, see Figure 1 and Table 2.

Pregnancy and postpartum period

The group of patients that reported an influence of pregnancy on social anxiety and avoidance was not entirely the same as the group of patients that reported an influence of the postpartum period. We only included data of patients who reported an influence of both the pregnancy and postpartum period in the statistical analysis. This made it possible to compare the social anxiety and avoidance scores of the period before pregnancy, the three trimesters of pregnancy, and the postpartum period.

We found a difference in social anxiety and social avoidance between the period before pregnancy, all trimesters of pregnancy, and the postpartum period for the first pregnancy ($p < 0.001$, $\chi^2 = 27.4$, $df = 4$, $n = 12$; $p < 0.001$, $\chi^2 = 24.2$, $df = 4$, $n = 10$). The same results were seen for the second pregnancy ($p = 0.003$, $\chi^2 = 15.8$, $df = 4$, $n = 11$; $p = 0.002$, $\chi^2 = 16.8$, $df = 4$, $n = 9$). Social anxiety and avoidance were highest before pregnancy and in the postpartum period. During pregnancy, patients reported less severe symptoms. For details see Figure 2 and Table 3.

Discussion

Most respondents did not report any influence of the female hormonal cycle on gSAD symptoms. However, the subgroup who did, reported more severe social anxiety and avoidance in the premenstrual period and, as opposed to our hypothesis less severe symptoms during pregnancy. In this subgroup, the vulnerability for the effects of the gonadal hormones on neurotransmitter systems associated with anxiety may be increased. If corroborated in further studies, it would be interesting to investigate whether this enhanced vulnerability is associated with fewer effects of treatments aimed at these neurotransmitter systems.

The literature on the influence of gonadal hormones on the course of anxiety disorders shows that these influences exist only in a subgroup of women. The premenstrual worsening of symptoms in a subgroup of women as shown in this study was also found in GAD, and obsessive compulsive disorder (OCD) (Hsiao et al., 2004; McLeod et al., 1993; Vulink et al., 2006; Labad et al., 2005; Williams and Koran, 1997). In one study on panic disorder (PD) an exacerbation of symptoms was also seen in the premenstrual period in some patients, whereas other studies in PD found no differences (Hsiao et al., 2004; Pigott, 1999; Breier et al., 1986; Kaspi et al., 1994; Stein et al., 1989; Cook et al., 1990; Cameron et al., 1988). The decrease of symptoms during pregnancy, as we found in gSAD, had previously been described in some, but not all, studies in PD (Hertzberg and Wahlbeck, 1999; Cohen et al., 1994; Klein et al., 1994; Altshuler et al., 1998). In OCD, the effects of pregnancy are not clear yet, as unchanged symptoms as well as worsening and improvement of symptoms were described (Vulink et al., 2006; Labad et al., 2005; Williams and Koran, 1997). In line with the present study, the same studies showed a postpartum increase of symptoms in a subgroup of OCD patients (Vulink et al., 2006; Labad et al., 2005; Williams and Koran, 1997).

There are also some limitations of the present study to discuss. As a result of the self-report survey and the retrospective nature of this study, the results are likely to be affected by a significant recall bias. However, in the absence of prospective studies, these retrospective data provide a first impression of the nature and extent of the influences of gonadal hormones in gSAD. Furthermore, no data were collected on the comorbidity between gSAD and premenstrual syndrome and premenstrual dysphoric disorder. Unfortunately, only 46% of the gSAD patients returned the surveys. This is mainly caused by the UMCU patients of whom we did not have recent addresses. In the LUMC group, of which we had recent addresses, the response rate was good (84%). However, we cannot rule out the possibility of responder bias, meaning that the women who believe in the influence of hormones on their gSAD symptoms or who experienced such an effect were more likely to respond. Our sample was not representative for the general population of female gSAD patients, as our respondents were prior participants in research studies in two university clinics. They were relatively highly educated and most of them had a job. Furthermore, we are not able to draw conclusions on differences in gSAD symptoms in OC use, lactation, and menopause, because of the small groups that were analyzed. Also the sub-analyses were carried out with very small sample sizes and therefore should be carefully interpreted. Other limitations are that there are many factors that could have influenced the gSAD symptoms besides the gonadal

hormones. For example, during pregnancy and in the post partum period life changes dramatically, which we did not take into account in the present study.

The clinician should take into account that there probably is at least a subgroup of female gSAD patients whose symptoms are influenced by fluctuations in female gonadal hormones: they have more severe symptoms premenstrually and less severe symptoms during pregnancy. Prospective studies will have to elucidate how large this proportion is and how much influence the gonadal hormones have on symptoms and treatment effects. Ultimately, this may lead to more tailor made treatments for gSAD patients.

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References

- Altshuler, L. L., Hendrick, V., and Cohen, L. S. (1998). Course of mood and anxiety disorders during pregnancy and the postpartum period. *J.Clin.Psychiatry*, 59 Suppl 2, 29-33.
- Brambilla, F., Biggio, G., Pisu, M. G., Bellodi, L., Perna, G., Bogdanovich-Djukic, V. et al. (2003). Neurosteroid secretion in panic disorder. *Psychiatry Res.*, 118, 107-116.
- Breier, A., Charney, D. S., and Heninger, G. R. (1986). Agoraphobia with panic attacks. Development, diagnostic stability, and course of illness. *Arch.Gen.Psychiatry*, 43, 1029-1036.
- Cameron, O. G., Kuttesch, D., McPhee, K., and Curtis, G. C. (1988). Menstrual fluctuation in the symptoms of panic anxiety. *J.Affect.Disord.*, 15, 169-174.
- Cohen, L. S., Sichel, D. A., Dimmock, J. A., and Rosenbaum, J. F. (1994). Postpartum course in women with preexisting panic disorder. *J.Clin.Psychiatry*, 55, 289-292.
- Cook, B. L., Noyes, R., Jr., Garvey, M. J., Beach, V., Sobotka, J., and Chaudhry, D. (1990). Anxiety and the menstrual cycle in panic disorder. *J.Affect.Disord.*, 19, 221-226.
- Fehm, L., Pelissolo, A., Furmark, T., and Wittchen, H. U. (2005). Size and burden of social phobia in Europe. *Eur.Neuropsychopharmacol.*, 15, 453-462.
- Hertzberg, T. and Wahlbeck, K. (1999). The impact of pregnancy and puerperium on panic disorder: a review. *J.Psychosom.Obstet.Gynaecol.*, 20, 59-64.
- Hsiao, M. C., Hsiao, C. C., and Liu, C. Y. (2004). Premenstrual symptoms and premenstrual exacerbation in patients with psychiatric disorders. *Psychiatry Clin.Neurosci.*, 58, 186-190.
- Kaspi, S. P., Otto, M. W., Pollack, M. H., Eppinger S., and Rosenbaum, J. F. (1994). Premenstrual exacerbation of symptoms in women with panic disorder. *J.Anxiety.Disord.* 8(2), 131-138.
- Keller, M. B. (2003). The lifelong course of social anxiety disorder: a clinical perspective. *Acta Psychiatr.Scand. Suppl*, 85-94.
- Klein, D. F., Skrobala, A. M., and Garfinkel, R. S. (1994). Preliminary look at the effects of pregnancy on the course of panic disorder. *Anxiety*, 1, 227-232.
- Labad, J., Menchon, J. M., Alonso, P., Segalas, C., Jimenez, S., and Vallejo, J. (2005). Female reproductive cycle and obsessive-compulsive disorder. *J.Clin.Psychiatry*, 66, 428-435.
- McLeod, D. R., Hoehn-Saric, R., Foster, G. V., and Hipsley, P. A. (1993). The influence of premenstrual syndrome on ratings of anxiety in women with generalized anxiety disorder. *Acta Psychiatr.Scand.*, 88, 248-251.
- Pigott, T. A. (1999). Gender differences in the epidemiology and treatment of anxiety disorders. *J.Clin.Psychiatry*, 60 Suppl 18, 4-15.
- Rofe, Y., Blittner, M., and Lewin, I. (1993). Emotional experiences during the three trimesters of pregnancy. *J.Clin.Psychol.*, 49, 3-12.
- Stein, M. B., Schmidt, P. J., Rubinow, D. R., and Uhde, T. W. (1989). Panic disorder and the menstrual cycle: panic disorder patients, healthy control subjects, and patients with premenstrual syndrome. *Am.J.Psychiatry*, 146(10), 1299-1303.
- Van Vliet, I. M. and De Beurs, E. (2007). [The MINI-International Neuropsychiatric Interview. A brief structured diagnostic psychiatric interview for DSM-IV en ICD-10 psychiatric disorders]. *Tijdschr.Psychiatr.*, 49, 393-397.
- Vulink, N. C., Denys, D., Bus, L., and Westenberg, H. G. (2006). Female hormones affect symptom severity in obsessive-compulsive disorder. *Int.Clin.Psychopharmacol.*, 21, 171-175.
- Weinstock, L. S. (1999). Gender differences in the presentation and management of social anxiety disorder. *J.Clin.Psychiatry*, 60 Suppl 9, 9-13.
- Williams, K. E. and Koran, L. M. (1997). Obsessive-compulsive disorder in pregnancy, the puerperium, and the premenstruum. *J.Clin.Psychiatry*, 58, 330-334.
- Wittchen, H. U. and Fehm, L. (2003). Epidemiology and natural course of social fears and social phobia. *Acta Psychiatr.Scand.Suppl*, 4-18.

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 (α_2) 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 - β -(4-iodophenyl)-tropane (^{123}I - β -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 - β -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 α_2 -adrenergic autoreceptors, resulting in the stimulation of both noradrenergic and serotonergic pathways. It also blocks 5-HT₂ and 5-HT₃ 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 ascends 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 adrenocortical 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 from 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 5HT_{1A}-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 5HT_{2C}-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 Tillfors, 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 psychotropic 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 an 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-axis) 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

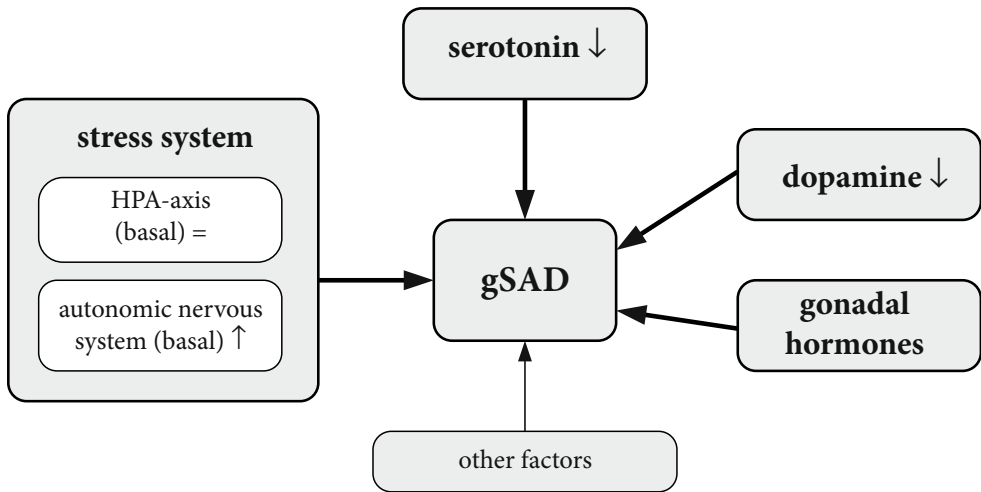
There are no studies that report on the influence of female gonadal hormones on gSAD symptoms as yet. Since the onset is often at puberty, and more women are affected and more severely than men, it might be expected that these hormones have an influence on gSAD.

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 made 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 makes 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 into account, but not the pulsatile release of cortisol, which could have influenced our results. Cortisol binds for 95% to large proteins, mostly cortisol binding globulin (CBG) and albumin. 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

- Alenina, N., Kikic, D., Todiras, M., Mosienko, V., Qadri, F., Plehm, R. et al. (2009). Growth retardation and altered autonomic control in mice lacking brain serotonin. *Proc.Natl.Acad.Sci.U.S.A.*, 106, 10332-10337.
- Argyropoulos, S. V., Hood, S. D., Adrover, M., Bell, C. J., Rich, A. S., Nash, J. R. et al. (2004). Tryptophan depletion reverses the therapeutic effect of selective serotonin reuptake inhibitors in social anxiety disorder. *Biol.Psychiatry*, 56, 503-509.
- Audero, E., Coppi, E., Mlinar, B., Rossetti, T., Caprioli, A., Banchaabouchi, M. A. et al. (2008). Sporadic autonomic dysregulation and death associated with excessive serotonin autoinhibition. *Science*, 321, 130-133.
- Benjamin, J., Li, L., Patterson, C., Greenberg, B. D., Murphy, D. L., and Hamer, D. H. (1996). Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. *Nat.Genet.*, 12, 81-84.
- Birbaumer, N., Grodd, W., Diedrich, O., Klose, U., Erb, M., Lotze, M. et al. (1998). fMRI reveals amygdala activation to human faces in social phobics. *Neuroreport*, 9, 1223-1226.
- Bouwer, C. and Stein, D. J. (1998). Hyperresponsivity to nicotinic acid challenge in generalized social phobia: a pilot study. *Eur.Neuropsychopharmacol.*, 8, 311-313.
- Condren, R. M., O'Neill, A., Ryan, M. C., Barrett, P., and Thakore, J. H. (2002). HPA axis response to a psychological stressor in generalised social phobia. *Psychoneuroendocrinology*, 27, 693-703.
- Demling, J., Beyer, S., and Kornhuber, J. (2009). To sweat or not to sweat? A hypothesis on the effects of venlafaxine and SSRIs. *Med.Hypotheses*.
- Ebstein, R. P., Nemanov, L., Klotz, I., Gritsenko, I., and Belmaker, R. H. (1997). Additional evidence for an association between the dopamine D4 receptor (D4DR) exon III repeat polymorphism and the human personality trait of Novelty Seeking. *Mol.Psychiatry*, 2, 472-477.
- Ebstein, R. P., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D. et al. (1996). Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. *Nat.Genet.*, 12, 78-80.
- Furlan, P. M., DeMartinis, N., Schweizer, E., Rickels, K., and Lucki, I. (2001). Abnormal salivary cortisol levels in social phobic patients in response to acute psychological but not physical stress. *Biol.Psychiatry*, 50, 254-259.
- Furmark, T., Tillfors, M., Garpenstrand, H., Marteinsdottir, I., Langstrom, B., Oreland, L. et al. (2004). Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neurosci.Lett.*, 362, 189-192.
- Furmark, T., Tillfors, M., Marteinsdottir, I., Fischer, H., Pissioti, A., Langstrom, B. et al. (2002). Common changes in cerebral blood flow in patients with social phobia treated with citalopram or cognitive-behavioral therapy. *Arch.Gen.Psychiatry*, 59, 425-433.
- Gerlach, A. L., Wilhelm, F. H., and Roth, W. T. (2003). Embarrassment and social phobia: the role of parasympathetic activation. *J.Anxiety Disord.*, 17, 197-210.
- Grossman, P., Wilhelm, F. H., Kawachi, I., and Sparrow, D. (2001). Gender differences in psychophysiological responses to speech stress among older social phobics: congruence and incongruence between self-evaluative and cardiovascular reactions. *Psychosom.Med.*, 63, 765-777.
- Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D. et al. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science*, 297, 400-403.
- Heinz, A., Braus, D. F., Smolka, M. N., Wrase, J., Puls, I., Hermann, D. et al. (2005). Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nat.Neurosci.*, 8, 20-21.
- Hollander, E., Kwon, J., Weiller, F., Cohen, L., Stein, D. J., DeCaria, C. et al. (1998). Serotonergic function in social phobia: comparison to normal control and obsessive-compulsive disorder subjects. *Psychiatry Res.*, 79, 213-217.
- Ipsen, J. C., Kariuki, C. M., and Stein, D. J. (2008). Pharmacotherapy for social anxiety disorder: a systematic review. *Expert.Rev.Neurother.*, 8, 235-257.
- Johnson, M. R., Lydiard, R. B., Zealberg, J. J., Fossey, M. D., and Ballenger, J. C. (1994). Plasma and CSF HVA levels in panic patients with comorbid social phobia. *Biol.Psychiatry*, 36, 425-427.

- Kendler, K. S., Myers, J., Prescott, C. A., and Neale, M. C. (2001). The genetic epidemiology of irrational fears and phobias in men. *Arch.Gen.Psychiatry*, 58, 257-265.
- Kendler, K. S., Neale, M. C., Kessler, R. C., Heath, A. C., and Eaves, L. J. (1992). The genetic epidemiology of phobias in women. The interrelationship of agoraphobia, social phobia, situational phobia, and simple phobia. *Arch.Gen.Psychiatry*, 49, 273-281.
- Kummer, A., Cardoso, F., and Teixeira, A. L. (2008). Frequency of social phobia and psychometric properties of the Liebowitz social anxiety scale in Parkinson's disease. *Mov Disord.*, 23, 1739-1743.
- Laederach-Hofmann, K., Mussgay, L., Buchel, B., Widler, P., and Ruddel, H. (2002). Patients with erythrophobia (fear of blushing) show abnormal autonomic regulation in mental stress conditions. *Psychosom.Med.*, 64, 358-365.
- Lanfumey, L., Mongeau, R., Cohen-Salmon, C., and Hamon, M. (2008). Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. *Neurosci.Biobehav.Rev.*, 32, 1174-1184.
- Lanzenberger, R. R., Mitterhauser, M., Spindelegger, C., Wadsak, W., Klein, N., Mien, L. K. et al. (2007). Reduced serotonin-1A receptor binding in social anxiety disorder. *Biol.Psychiatry*, 61, 1081-1089.
- Ledoux, J. E. (1998). *The Emotional Brain: The Mysterious Underpinnings of Emotional Life*. Simon and Schuster.
- Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S. et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274, 1527-1531.
- Li, S., Tan, J., Qian, M., Liu, X. (2008). Continual training of attentional bias in social anxiety. *Behav. Res. Ther.* 46(8), 905-912.
- Lorberbaum, J. P., Kose, S., Johnson, M. R., Arana, G. W., Sullivan, L. K., Hamner, M. B. et al. (2004). Neural correlates of speech anticipatory anxiety in generalized social phobia. *Neuroreport*, 15, 2701-2705.
- Martel, F. L., Hayward, C., Lyons, D. M., Sanborn, K., Varady, S., and Schatzberg, A. F. (1999). Salivary cortisol levels in socially phobic adolescent girls. *Depress.Anxiety*, 10, 25-27.
- Meijer, O. C. and De Kloet, E. R. (1998). Corticosterone and serotonergic neurotransmission in the hippocampus: functional implications of central corticosteroid receptor diversity. *Crit Rev.Neurobiol.*, 12, 1-20.
- Mueller, A., Brocke, B., Fries, E., Lesch, K. P., and Kirschbaum, C. (2009). The role of the serotonin transporter polymorphism for the endocrine stress response in newborns. *Psychoneuroendocrinology*.
- Noble, E. P., Ozkaragoz, T. Z., Ritchie, T. L., Zhang, X., Belin, T. R., and Sparkes, R. S. (1998). D2 and D4 dopamine receptor polymorphisms and personality. *Am.J.Med.Genet.*, 81, 257-267.
- Ono, Y., Manki, H., Yoshimura, K., Muramatsu, T., Mizushima, H., Higuchi, S. et al. (1997). Association between dopamine D4 receptor (D4DR) exon III polymorphism and novelty seeking in Japanese subjects. *Am.J.Med.Genet.*, 74, 501-503.
- Potts, N. L., Davidson, J. R., Krishnan, K. R., Doraiswamy, P. M., and Ritchie, J. C. (1991). Levels of urinary free cortisol in social phobia. *J.Clin.Psychiatry*, 52 Suppl:41-2., 41-42.
- Rodrigues, S. M., Ledoux, J. E., and Sapolsky, R. M. (2009). The influence of stress hormones on fear circuitry. *Annu.Rev.Neurosci.*, 32, 289-313.
- Roelofs, K., Elzinga, B. M., and Rotteveel, M. (2005). The effects of stress-induced cortisol responses on approach-avoidance behavior. *Psychoneuroendocrinology*, 30, 665-677.
- Roelofs, K., van, P. J., Berretty, E., Jong, P., Spinhoven, P., and Elzinga, B. M. (2009). Hypothalamus-pituitary-adrenal axis hyperresponsiveness is associated with increased social avoidance behavior in social phobia. *Biol.Psychiatry*, 65, 336-343.
- Rowe, D. C., Stever, C., Gard, J. M., Cleveland, H. H., Sanders, M. L., Abramowitz, A. et al. (1998). The relation of the dopamine transporter gene (DAT1) to symptoms of internalizing disorders in children. *Behav. Genet.*, 28, 215-225.
- Sareen, J., Campbell, D. W., Leslie, W. D., Malisza, K. L., Stein, M. B., Paulus, M. P. et al. (2007). Striatal function in generalized social phobia: a functional magnetic resonance imaging study. *Biol.Psychiatry*, 61, 396-404.
- Schneier, F. R., Liebowitz, M. R., Abi-Dargham, A., Zea-Ponce, Y., Lin, S. H., and Laruelle, M. (2000). Low dopamine D(2) receptor binding potential in social phobia. *Am.J.Psychiatry*, 157, 457-459.

- Shah, S. G., Klumpp, H., Angstadt, M., Nathan, P. J., and Phan, K. L. (2009). Amygdala and insula response to emotional images in patients with generalized social anxiety disorder. *J.Psychiatry Neurosci.*, 34, 296-302.
- Soderpalm, B., Lof, E., and Ericson, M. (2009). Mechanistic studies of ethanol's interaction with the mesolimbic dopamine reward system. *Pharmacopsychiatry*, 42 Suppl 1, S87-S94.
- Stein, D. J., Westenberg, H. G., and Liebowitz, M. R. (2002). Social anxiety disorder and generalized anxiety disorder: serotonergic and dopaminergic neurocircuitry. *J.Clin.Psychiatry*, 63 Suppl 6, 12-19.
- Stein, M. B., Asmundson, G. J., and Chartier, M. (1994). Autonomic responsivity in generalized social phobia. *J.Affect.Disord.*, 31, 211-221.
- Stein, M. B., Chartier, M. J., Kozak, M. V., King, N., and Kennedy, J. L. (1998). Genetic linkage to the serotonin transporter protein and 5HT2A receptor genes excluded in generalized social phobia. *Psychiatry Res.*, 81, 283-291.
- Stein, M. B., Goldin, P. R., Sareen, J., Zorrilla, L. T., and Brown, G. G. (2002). Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Arch.Gen.Psychiatry*, 59, 1027-1034.
- Stein, M. B., Heuser, I. J., Juncos, J. L., and Uhde, T. W. (1990). Anxiety disorders in patients with Parkinson's disease. *Am.J.Psychiatry*, 147, 217-220.
- Stein, M. B., Tancer, M. E., and Uhde, T. W. (1992). Heart rate and plasma norepinephrine responsivity to orthostatic challenge in anxiety disorders. Comparison of patients with panic disorder and social phobia and normal control subjects. *Arch.Gen.Psychiatry*, 49, 311-317.
- Strobel, A., Wehr, A., Michel, A., and Brocke, B. (1999). Association between the dopamine D4 receptor (DRD4) exon III polymorphism and measures of Novelty Seeking in a German population. *Mol.Psychiatry*, 4, 378-384.
- Talarovicova, A., Krskova, L., and Kiss, A. (2007). Some assessments of the amygdala role in suprahypothalamic neuroendocrine regulation: a minireview. *Endocr.Regul.*, 41, 155-162.
- Tancer, M. E., Mailman, R. B., Stein, M. B., Mason, G. A., Carson, S. W., and Golden, R. N. (1994). Neuroendocrine responsivity to monoaminergic system probes in generalized social phobia. *Anxiety*, 1, 216-223.
- Tiihonen, J., Kuikka, J., Bergstrom, K., Lepola, U., Koponen, H., and Leinonen, E. (1997). Dopamine reuptake site densities in patients with social phobia. *Am.J.Psychiatry*, 154, 239-242.
- Tillfors, M. (2004). Why do some individuals develop social phobia? A review with emphasis on the neurobiological influences. *Nord.J.Psychiatry*, 58, 267-276.
- Tillfors, M., Furmark, T., Marteinsdottir, I., Fischer, H., Pissiota, A., Langstrom, B. et al. (2001). Cerebral blood flow in subjects with social phobia during stressful speaking tasks: a PET study. *Am.J.Psychiatry*, 158, 1220-1226.
- Uhde, T. W., Tancer, M. E., Gelernter, C. S., and Vittone, B. J. (1994). Normal urinary free cortisol and post-dexamethasone cortisol in social phobia: comparison to normal volunteers. *J.Affect.Disord.*, 30, 155-161.
- Van Praag, H. M. (1996). Faulty cortisol/serotonin interplay. Psychopathological and biological characterization of a new, hypothetical depression subtype (SeCA depression). *Psychiatry Res.*, 65, 143-157.

Nederlandse samenvatting en discussie

Samenvatting

In dit proefschrift wordt onderzoek beschreven naar de betrokkenheid van diverse neurobiologische systemen in de hersenen bij gegeneraliseerde sociale angststoornis.

In **hoofdstuk 1** wordt het proefschrift ingeleid. Sociale angststoornis (SAD)¹, ook wel sociale fobie genoemd, wordt gekenmerkt door een aanhoudende angst voor sociale situaties waarin de persoon in contact komt met mensen of mogelijke kritiek van anderen. Voorbeelden van dit soort situaties zijn: het voeren van een gesprek, het bijwonen van een vergadering, het geven van een presentatie, op visite gaan, koffie drinken in gezelschap, telefoneren in gezelschap en het geven van de eigen mening of kritiek. Blootstelling aan de gevreesde situaties lokt angst uit, die vaak gepaard gaat met lichamelijke angstverschijnselen zoals blozen, transpireren, trillen en/of hartkloppingen. De angst kan zo hevig zijn dat er een paniekaanval ontstaat. Door deze klachten ontstaat anticipatieangst en vermijdingsgedrag ten aanzien van de sociale situaties. De klachten gaan meestal gepaard met veel schaamte. De symptomen ontstaan in het algemeen in de puberteit of al daarvoor. Er kunnen twee subtypen van SAD onderscheiden worden: het specifieke subtype (sSAD) en het gegeneraliseerde subtype (gSAD). In dit proefschrift hebben we ervoor gekozen het gegeneraliseerde subtype te onderzoeken, omdat dat het meest invaliderende, ernstigste en meest volledige type is en alle aspecten van sociale angst vertoont. gSAD is geassocieerd met verminderd functioneren in het sociale leven, het gezinsleven en op het werk. Een Canadese studie naar het voorkomen van gSAD toont aan dat gedurende het leven 5.9 % van de bevolking op een bepaald moment aan gSAD lijdt. Conform de Nederlandse Multidisciplinaire Richtlijnen bestaat de behandeling van gSAD uit cognitieve gedragstherapie (een vorm van kortdurende psychotherapie), medicatie, of een combinatie van beide. Als wordt gekozen voor medicatie zijn selectieve serotonine heropname remmers (SSRI's) of serotonine-noradrenaline heropname remmers (SNRI's) de eerst aangewezen middelen.

De neurobiologische oorzaken van gSAD zijn niet precies bekend. Het is van belang deze te onderzoeken om de stoornis en het ontstaan ervan beter te begrijpen en zo ook nieuwe en effectieve behandelmethoden te kunnen ontwikkelen. Onderzoek naar de neurobiologie van andere angst- en stemmingsstoornissen toonde de mogelijke betrokkenheid van diverse neurotransmitter systemen in de hersenen aan. Voorbeelden hiervan zijn het serotonerge en het dopaminerge systeem. Serotonine en dopamine zijn neurotransmitters (boodschapperstoffen) die de informatieoverdracht tussen zenuwcellen verzorgen. Daarnaast is er het stress-systeem, dat bestaat uit het autonome zenuwstelsel (ANS) en de hypothalamus-hypofyse-bijnier-as (HPA-as), en dat neurotransmittersystemen in de hersenen moduleert. Bij stress wordt het hormoon CRH (corticotropin releasing hormone) in de hersenen afgegeven. Dit hormoon activeert zowel het ANS als de HPA-as. Bij stress of gevaar zorgt het ANS ervoor dat, via de uitscheiding van adrenaline en noradrenaline, het lichaam in directe staat van paraatheid wordt gebracht. De HPA-

¹ In deze Nederlandse samenvatting worden de Engelse afkortingen gebruikt conform het Engelstalige deel van dit proefschrift

as wordt ook geactiveerd door stress, waardoor de bijnieren worden gestimuleerd tot de afgifte van het stresshormoon cortisol in het bloed. Verder beïnvloeden vrouwelijke geslachtshormonen angst en stemming en zouden dus betrokken kunnen zijn bij affectieve stoornissen. Het doel van dit proefschrift is om de betrokkenheid van de bovengenoemde systemen bij de neurobiologie van gSAD te onderzoeken. gSAD en paniekstoornis (PD) behoren tot de meest voorkomende angststoornissen. Hoewel ze verschillende primaire kenmerken hebben, PD wordt gekenmerkt door spontane paniekaanvallen en gSAD door de angst om door anderen bekritiseerd te worden, suggereren epidemiologische, farmacologische, genetische en neurobiologische onderzoeken dat beide angststoornissen elkaar op neurobiologisch gebied overlappen. In **hoofdstuk 2** hebben we de gedragsmatige en biologische effecten van een acute serotonerge provocatietest bij zeven (vijf mannen en twee vrouwen) patiënten met gSAD, PD en gezonde controles direct met elkaar vergeleken. Deze provocatietest werd verricht door 0.1 mg/kg meta-chlorophenylpiperazine (m-CPP), een stof die het serotonerge systeem stimuleert, maar die ook een gering effect heeft op het adrenerge en dopaminerge systeem, snel in een bloedvat toe te dienen. De deelnemers hadden geen andere psychiatrische of lichamelijke ziekten. Na de provocatietest werd het effect op het angstniveau van de deelnemers gemeten en werd bepaald of er een paniekaanval was opgetreden. Daarnaast werden de temperatuur, de bloeddruk en de hartfrequentie gemeten en werden cortisol en groeihormoon in het bloed bepaald. Paniekaanvallen uitgelokt door m-CPP toediening bleken vaker voor te komen bij mensen met PD (85%) dan bij mensen met gSAD (14%) en gezonde controles (0%). De angstscores waren hoger bij patiënten met PD dan in de andere twee groepen. Er waren geen verschillen in de groepen wat betreft de temperatuur, bloeddruk, hartfrequentie, of hormoon spiegels. De eerste conclusie op basis van deze studie was dat de verschillen in respons op m-CPP suggereren dat PD en gSAD verschillende psychiatrische stoornissen zijn op neurobiologisch niveau. De tweede conclusie was dat patiënten met gSAD anders reageren op deze serotonerge provocatietest dan gezonde controles. Hiermee werd aangetoond dat het serotonerge systeem een rol speelt in de neurobiologie van gSAD. We hebben dit verder geëxploreerd in het onderzoek dat in hoofdstuk 3 wordt beschreven.

In **hoofdstuk 3** beschrijven we een studie waarin we de binding van ^{123}I - β -(4-iodophenyl)-tropane (^{123}I - β -CIT) aan de serotonine en dopamine transporteiwitten met behulp van SPECT scans onderzochten. Bij een SPECT scan (afkorting van Single Photon Emission Computed Tomography) wordt gebruik gemaakt van radioactief gelabelde stoffen waardoor een driedimensioneel beeld gemaakt wordt. Doordat ^{123}I - β -CIT zich selectief bindt aan de serotonine en dopamine transporteiwitten kan een afbeelding worden verkregen van de hoeveelheid en verdeling hiervan in de hersenen. Om de binding aan de transporteiwitten voor serotonine vast te leggen werd 4 uur na toediening van de ^{123}I - β -CIT een SPECT scan gemaakt, om de binding aan de transporteiwitten voor dopamine vast te leggen na 24 uur. Twaalf patiënten met gSAD (5 vrouwen en 7 mannen), die nog nooit psychiatrische medicatie hadden gebruikt en 12 op leeftijd en geslacht gemaakte gezonde vrijwilligers werden onderzocht. We vonden hogere binding van ^{123}I - β -CIT aan de transporteiwitten voor serotonine in de thalamus (een hersenstructuur die een

verbindingstation vormt tussen de informatie die binnenkomt vanuit het lichaam en de hogere hersengebieden) en een hogere binding van ^{123}I - β -CIT aan de transporteiwitten voor dopamine in het striatum (een hersenstructuur die onder andere geactiveerd is bij belonende stimuli) bij gSAD patiënten vergeleken met gezonde vrijwilligers. Dit onderzoek levert direct bewijs dat er afwijkingen zijn in zowel het serotonerge als het dopaminerge systeem bij patiënten met gSAD.

De effectiviteit van mirtazapine op de symptomen van gSAD werd bestudeerd in een onderzoek dat beschreven is in **hoofdstuk 4**. Mirtazapine is een antidepressivum dat het serotonerge systeem stimuleert via stimulatie van het noradrenerge systeem. Mirtazapine beïnvloedt ook het histaminerge systeem. Studies met deze stof bij andere angststoornissen suggereerden dat mirtazapine angstverlagend werkt. We onderzochten de effecten van mirtazapine 30 mg per dag gedurende 12 weken bij veertien patiënten met gSAD zonder andere psychiatrische stoornis. Twaalf patiënten maakten het onderzoek af. Twee patiënten (14.3%) vielen uit omdat ze teveel last van bijwerkingen hadden. In het algemeen werd mirtazapine goed verdragen. Vijf van de twaalf patiënten werden geclassificeerd als responders op de behandeling, wat wil zeggen dat ze een afname hadden op de Liebowitz Sociale Angst Schaal (LSAS) van 40% of meer. De gemiddelde LSAS score in de hele groep patiënten nam significant af. Dit onderzoek suggereert de betrokkenheid van serotonerge, noradrenerge en mogelijk histaminerge systemen in gSAD.

In **hoofdstuk 5** hebben we onderzoek naar zowel de HPA-as activiteit als de activiteit van het autonome zenuwstelsel (ANS) beschreven bij patiënten met gSAD in basale omstandigheden en na toediening van 0.5 mg dexamethason. Met dexamethason kan de remming van het stress-systeem getest worden. Om ervan verzekerd te zijn dat de verzameling van de gegevens stressvrij verliep hebben we speeksel in plaats van bloed verzameld waaruit cortisol en speeksel alpha-amylase (sAA) werden bepaald. (Bloed prikken levert stress op wat ervoor zorgt dat het stress-systeem ongewenst wordt geactiveerd. Speeksel wordt verzameld door het kauwen op een watje en is niet stressvol). Cortisol is een al langer bestaande maat voor HPA-as activiteit. sAA is een relatieve nieuwe marker voor het autonome zenuwstelsel. Drieënveertig onbehandelde gSAD patiënten zonder comorbiditeit werden vergeleken met 43 op leeftijd en geslacht geselecteerde gezonde controles op sAA en speeksel cortisol in niet-stressvolle omstandigheden, gemeten op verschillende tijdstippen van de dag. Aan het eind van de eerste meetdag werd dexamethason ingenomen, en de volgende dag werden sAA en cortisol opnieuw op 2 tijdstippen gemeten. De resultaten waren dat patiënten met gSAD significant hogere sAA waarden over de dag hadden, zowel vóór als na toediening van dexamethason. Er werden geen verschillen gevonden tussen de groepen wat betreft de cortisolwaarden. Dit betekent dat bij gSAD onder basale, niet-stressvolle, omstandigheden hyperactiviteit van het autonome zenuwstelsel (ANS) aanwezig is. Bovendien hebben we gevonden dat de HPA-as activiteit niet parallel loopt aan de activiteit van het ANS, maar gelijk blijft aan die van de gezonde vrijwilligers. De hyperactiviteit van het ANS komt overeen met de klinisch geobserveerde lichamelijke symptomen van hyperarousal bij gSAD zoals trillen, blozen en transpireren.

Hoofdstuk 6 beschrijft de wisselwerking tussen het serotonerge systeem en het stress-systeem bij gSAD. Twee groepen met negen paarsgewijs op leeftijd en geslacht gemaakte patiënten met gSAD, die succesvol behandeld waren met een SSRI (citalopram), ondergingen een tryptofaan-depletietest (TD) of een placeboconditie. Een TD procedure zorgt ervoor dat er tijdelijk minder serotonine beschikbaar is, en dus dat het serotonerge systeem minder actief is. Om het stress-systeem te activeren werd deze TD procedure of de placebo procedure gecombineerd met een 'public speaking task', waarbij de deelnemer een voordracht moest houden voor een gefingeerd publiek. Om de stress te verhogen werd voorgewend dat deze voordracht werd opgenomen op video en er werd verteld dat er een beoordeling op zou volgen. In het speeksel werd sAA gemeten als maat voor het ANS en speeksel cortisol als maat voor de HPA-as. De resultaten waren dat de na de 'public speaking task' de TD groep een significant hogere sAA respons had dan de placebo groep. Dit betekent dat er sprake is van hyperresponsiviteit van het ANS in deze groep. Er werden geen verschillen gezien in cortisolrespons tussen de groepen. Deze resultaten suggereren dat er bij gSAD een ontregeling is in ANS functie en niet in HPA-as functie. Aangezien dit werd gevonden na een TD, betekent dat mogelijk dat het serotonerge systeem een modulerende rol speelt in de hyperresponsiviteit van het ANS.

In **hoofdstuk 7** staat een studie beschreven die tot doel had de rol van de vrouwelijke geslachtshormonen in gSAD te exploreren. We recruteerden vrouwelijke patiënten met gSAD die voorheen hadden meegewerkt aan onze onderzoeksprojecten in het Universitair Medisch Centrum Utrecht en het Leids Universitair Medisch Centrum. Zesenvertig procent van 140 benaderde vrouwen met gSAD retourneerden de enquête met vragen over de invloed van menarche (allereerste menstruatie), menstruele cyclus, gebruik van orale anticonceptie, zwangerschap, borstvoeding, periode na de bevalling en de overgang, op gSAD symptomen. De meeste respondenten rapporteerden geen invloed van deze hormonale fasen op hun sociale angstklachten. Maar, in de subgroep van vrouwen die wel invloed hadden gerapporteerd, werden statistische verschillen gevonden op hun gerapporteerde angstverschijnselen gedurende de menstruele cyclus en de zwangerschap. In de premenstruele periode rapporteerden deze vrouwen ernstiger gSAD symptomen. Tijdens de zwangerschap werden de symptomen minder, maar in de periode na de bevalling werden de symptomen weer net zo ernstig als vóór de zwangerschap. Dus in een subgroep van vrouwen werd een invloed bemerkt van de vrouwelijke geslachtshormonen op gSAD symptomen gedurende de menstruele cyclus en de zwangerschap.

Discussie

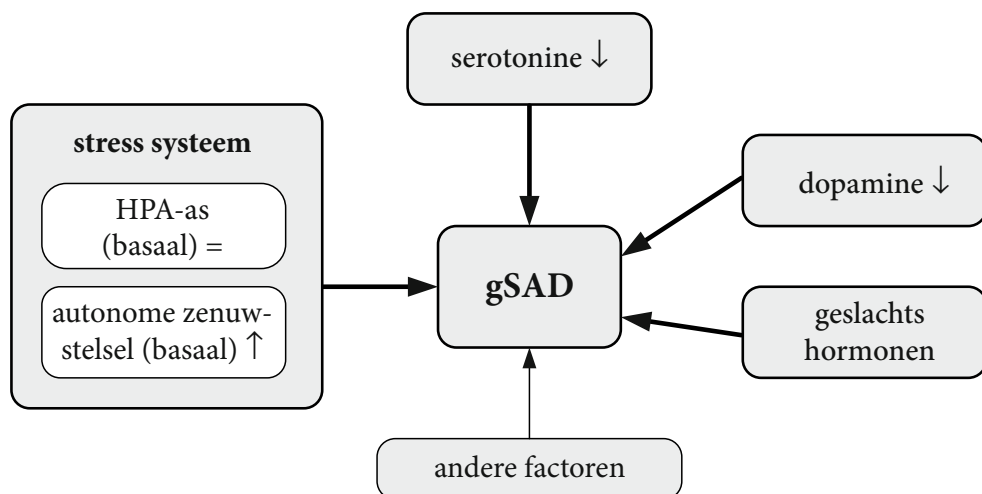
Het resultaat van de m-CPP provocatie test in **hoofdstuk 2** waarbij de patiënten met gSAD een sterkere reactie laten zien dan de gezonde controles is mogelijk het resultaat van verlaagde serotonerge activiteit. De verhoogde binding van ^{123}I - β -CIT aan de serotonine transporteiwitten in de thalamus in **hoofdstuk 3** kan het resultaat zijn van verminderde serotonine niveaus rondom de transporteiwitten of van een groter aantal transporteiwitten met als resultaat minder serotonine in de omgeving. Onze

hypothese, voortkomend uit beide studies, is dat gSAD geassocieerd is met verlaagde serotonerge activiteit. Deze hypothese wordt onder meer gesteund door de effectiviteit van SSRI's bij patiënten met gSAD.

Met betrekking tot de hogere binding van ^{123}I - β -CIT aan de dopamine transporteiwitten in het striatum in gSAD, zoals beschreven in **hoofdstuk 3**, gelden dezelfde verklaringsmogelijkheden als hierboven beschreven voor de serotonine transporteiwitten. Wij hypothetiseren dat er bij gSAD sprake is van verminderde dopaminerge activiteit, hetgeen bijvoorbeeld ook zou passen bij het toegenomen voorkomen van gSAD bij (voorafgaand aan de manifestatie van) de ziekte van Parkinson.

Uit **hoofdstuk 4, 5 en 6** kan geconcludeerd worden dat het noradrenerge systeem/ANS hyperactief is bij gSAD, terwijl de HPA-as functie niet afwijkt van gezonde controles.

Uit **hoofdstuk 7** kan de conclusie worden getrokken dat een subgroep van vrouwen met gSAD kwetsbaar lijkt te zijn voor de invloeden van vrouwelijke geslachtshormonen.



Op grond van onze conclusies komen we tot het bovenstaande hypothetische model dat de neurobiologie van gSAD schematisch weergeeft. Wij denken dat er sprake is van verminderd functioneren van zowel het serotonerge als het dopaminerge systeem, dat er sprake is van verhoogde activiteit van het autonome zenuwstelsel en dat de HPA-as functie niet synchroon loopt met de verhoogde activiteit van het autonome zenuwstelsel. We denken ook dat er aanwijzingen zijn voor de modulerende rol van vrouwelijke geslachtshormonen bij gSAD bij een subgroep van vrouwen.

Conclusie: de onderzoeken beschreven in dit proefschrift zijn een belangrijke aanvulling op de inzichten wat betreft de rol van het serotonerge en dopaminerge systeem, het stress-systeem bestaand uit het autonome zenuwstelsel en de hypothalamus-hypofyse-bijnier-as, en de vrouwelijke geslachtshormonen in de neurobiologie van de gegeneraliseerde sociale angststoornis.

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List of publications

Mirtazapine in social anxiety disorder: a pilot study. Van Veen, J.F., Van Vliet, I.M., Westenberg, H.G.M. (2002). *Int. Clin. Psychopharmacol.* 17(6), 315-317

Behavioural effects of rapid intravenous administration of meta-chlorophenylpiperazine (m-CPP) in patients with generalized social anxiety disorder, panic disorder and healthy controls. Van Veen, J.F., Van der Wee, N.J.A., Fiselier, J., Van Vliet, I.M., Westenberg, H.G.M. (2007). *Eur. Neuropsychopharmacol.* 17(10), 637-642.

Increased serotonin and dopamine transporter binding in psychotropic medication-naïve patients with generalized social anxiety disorder shown by ¹²³I-beta-(4-iodophenyl)-tropane SPECT. Van der Wee, N.J.A., Van Veen, J.F., Stevens, H., Van Vliet, I.M., Van Rijk, P.P., Westenberg, H.G.M. (2008). *J. Nucl. Med.* 49(5), 757-763.

Elevated alpha-amylase but not cortisol in generalized social anxiety disorder. Van Veen, J.F., Van Vliet, I.M., Derijk, R.H., Van Pelt, J., Mertens, B., Zitman, F.G. (2008). *Psychoneuroendocrinology* 33 (10), 1313-1321.

Tryptophan depletion affects the autonomic stress response in generalized social anxiety disorder. Van Veen, J.F., Van Vliet, I.M., Derijk, R.H., Van Pelt, J., Mertens, B., Fekkes, D., Zitman, F.G. (2009) *Psychoneuroendocrinology* 34(10), 1590-1594.

The effects of female reproductive hormones in generalized social anxiety disorder. Van Veen, J.F., Jonker, B.W., Van Vliet, I.M., Zitman, F.G. (2009) *Int. J. Psychiatry Med.* 39(3), 283-295.

Mirtazapine in generalized social anxiety disorder: a randomized, double-blind, placebo-controlled study. Schutters, S.I.J., Van Megen, H.J.G.M., Van Veen, J.F., Denys, D.A.J.P., Westenberg, H.G.M. (2010). *Int. Clin. Psychopharmacol.* Accepted for publication.

The combination of paroxetine and mirtazapine in generalized social anxiety disorder – mirtazapine addition in social anxiety disorder -. Schutters, S.I.J., Van Megen, H.J.G.M., Van Veen, J.F., Schruers, K.R.J., Westenberg, H.G.M. Submitted.

In gesprek over: Sociale Fobie. Van Veen, J.F., Van Vliet, I.M., Van Balkom, A.J.L.M. (2004) Nederlandse Vereniging voor Psychiatrie.

Curriculum Vitae

Frederieke van Veen werd geboren op 4 juni 1974 in Utrecht. Na het behalen van haar gymnasium diploma aan het College Blaucapel in Utrecht begon zij in 1992 aan de studie geneeskunde aan de Rijksuniversiteit van Utrecht. In 1996 deed zij een wetenschappelijke stage van een jaar naar 'Hormonale veranderingen bij vrouwen in de menopauze en de neiging tot agressie' bij mevr. prof. dr. P.T. Cohen-Kettenis in het Academisch Ziekenhuis Utrecht. In 1997 behaalde zij het doctoraalexamen en na het doen van de coschappen in 1999 het artsexamen. Aansluitend werkte zij ruim twee jaar als arts-onderzoeker bij de angstonderzoeksgroep van de afdeling psychiatrie in het Universitair Medisch Centrum Utrecht, waar ze begon met het onderzoek naar de neurobiologie van gegeneraliseerde sociale angststoornis. Zij werd hierin begeleid door mevr. dr. I.M. van Vliet en prof. dr. H.G.M. Westenberg. Vanaf 2002 zette ze het onderzoek naar gegeneraliseerde sociale angststoornis voort op de afdeling psychiatrie van het LUMC, eerst als arts-onderzoeker en later als aiosko (assistent in opleiding tot specialist en klinisch onderzoeker). Ze startte in oktober 2004 met de eerste opleidingsstage van de opleiding psychiatrie van LUMC/Rivierduinen met prof. dr. F.G. Zitman als hoofdopleider en mevr. prof. dr. R.C. van der Mast als waarnemend opleider. Voor de opleiding psychiatrie werkte ze een jaar in het LUMC en de overige jaren bij GGZ Leiden van Rivierduinen. Ze is momenteel werkzaam bij het Centrum Persoonlijkheidsstoornissen Jelgersma van GGZ Leiden en zal de opleiding psychiatrie naar verwachting in april 2011 afronden. Frederieke is getrouwd met Kees Holland en is de trotse moeder van 2 dochters, Rachel (4 jaar) en Sofie (2 jaar).

Abbreviations

ACTH	adrenocorticotrope hormone
ANS	autonomic nervous system
AUC	area under the curve
AVP	arginine-vasopressine
β -CIT	I- β -(4-iodophenyl)-tropane
BDI	Beck depression inventory
BMI	body mass index
BP	blood pressure
CAR	cortisol awakening rise
CBT	cognitive behavioural therapy
CGI	clinical global improvement scale
COMT	catechol-O-methyltransferase
CRH	corticotrophin releasing hormone
DAT	dopamine transporter
DSM	diagnostic and statistical manual of mental disorders
DST	dexamethasone suppression test
ER $_{\beta}$	estrogen receptor β
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GAD	generalized anxiety disorder
gSAD	generalized social anxiety disorder
GH	growth hormone
GR	glucocorticoid receptor
HDRS	Hamilton depression rating scale
HPA-axis	hypothalamic-pituitary-adrenal-axis
5HT	5-hydroxytryptamine = serotonin
5HTP	5-hydroxytryptophan
5HTT	serotonin transporter
5HTTLPR	serotonin linked polymorphic region polymorphism
LNAA	large neutral amino acid
LOCF	last observation carried forward
LSAS	Liebowitz social anxiety scale
LUMC	Leiden University Medical Center
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
m-CPP	metachlorophenylpiperazine
MR	mineralocorticoid receptor
MRI	magnetic resonance imaging

OC	oral contraceptives
OCD	obsessive compulsive disorder
PD	panic disorder
PET	positron emission tomography
POMS	profile of mood states
PSS	panic symptom scale
PTSD	posttraumatic stress disorder
ROI	region of interest
sAA	salivary alpha amylase
SAD	social anxiety disorder
SPECT	single photon emission computed tomography
SNRI	serotonin-noradrenaline reuptake inhibitor
sSAD	specific social anxiety disorder
SSRI	selective serotonin reuptake inhibitor
TD	tryptophan depletion
TRP	tryptophan
UMCU	University Medical Center Utrecht
VAS	visual analogue scale
VOI	volume of interest

