ON THE ASSOCIATION BETWEEN PANIC DISORDER AND AUTONOMIC REGULATION WITH SPECIAL FOCUS ON THE ROLES OF RESPIRATION AND ON THE CATECHOL-O-METHYLTRANSFERASE GENE

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Abstract

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Background and aims: Panic disorder is a psychiatric disorder characterized by sudden attacks of intense anxiety. It displays a lot of features suggesting that it may be associated with an underlying aberration in the autonomic regulation of heart activity and respiration: i) the attacks are often characterized by respiratory symptoms and symptoms from the heart, ii) the attacks can be elicited by respiratory stimulants, iii) between attacks, patients with panic disorder often display enhanced respiratory variability and reduced heart rate variability, and iv) patients with panic disorder display enhanced prevalence of respiratory disorders and enhanced mortality in cardiovascular disease. Addressing the reasons for these physiological aberrations may help in elucidating the pathophysiology underlying panic disorder, and shed light on why this disorder is associated with enhanced mortality in cardiovascular disease. Serotonin is believed to be a neurotransmitter of great importance for panic disorder, as well as for the regulation of respiration: one main purpose of the animal studies presented in this thesis hence was to increase our knowledge regarding the role of serotonin in respiratory regulation, the hypothesis being that aberrations in respiration may cause the anxiety attacks, and that serotonin-modulating drugs may prevent panic attacks partly by stabilizing the regulation of respiration. In the first part of the thesis, data is presented on the effects on respiration in freely moving rats of various serotonergic compounds. The second part of this thesis is focused on genetic variations that may be associated with panic disorder. Orexin is a neuropeptide of suggested importance for both respiratory regulation and arousal. We investigated two polymorphisms in the orexin receptors 1 and 2, HCRTR1 Ile408Val and HCRTR2 Val308Iso, in panic disorder patients and healthy controls**.** Catechol-O-methyltransferase (COMT) is an enzyme that degrades catecholamines such as dopamine and noradrenaline, and may thus be of importance for both autonomic control and psychiatric symptoms. The functional Val158Met polymorphism in this gene has been associated with panic disorder in several studies; in an attempt to replicate this finding, we genotyped this polymorphism in the same group of panic disorder patients. In a separate cohort, we also explored if the same polymorphism is associated with risk factors for cardiovascular disease. **Observations:** 1) Serotonin depletion with para-chlorophenylalanine *decreased* respiratory rate and *increased* respiratory variability. 2) Chronic treatment with serotonin reuptake inhibitors *increased* respiratory rate. 3) Acute treatment with serotonin reuptake inhibitors, as well as the serotonin releasing drugs d-fenfluramine and m-CPP, and the 5-HT1A antagonist WAY-100635, *decreased* respiratory rate. 4) The HCRTR2 Val308Iso polymorphism was significantly associated with panic disorder in women. 5) In line with previous studies in Caucasian samples, the COMT Val158 allele was significantly more frequent in PD patients than controls. 6) Met158 allele carriers displayed significantly higher waist-hip-ratio, sagittal diameter, systolic and diastolic blood pressure, and heart rate, than Val158 allele carriers in a population of healthy men. **Conclusions:** Our results suggest that serotonin exert a modulatory role on respiration, and support the notion that an influence on respiration may contribute both to the anxiogenic and the anti-panic effects of serotonergic drugs. The association between panic disorder and the hypocretin receptor-2 Val308Iso polymorphism is a novel finding in need of replication, whereas the association between panic disorder and the COMT Val158 allele can by now be regarded as confirmed. The association between the COMT Val158Met polymorphism and cardiovascular risk factors is of interest, but does not support the theory that this polymorphism contributes to the enhanced mortality in cardiovascular disease seen in panic disorder patients.

Key words: panic disorder – serotonin – respiration – polymorphism – COMT Val158Met – HCRTR2 G1246A – blood pressure – anthropometry

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This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I. **Annerbrink K**, Olsson M, Melchior LK, Hedner J, Eriksson E. Serotonin depletion increases respiratory variability in freely moving rats: implications for panic disorder. *International Journal of Neuropsychopharmacology*, Mar;6(1):51-6, 2003.

II. Olsson M, **Annerbrink K**, Bengtsson F, Hedner J, Eriksson E. Paroxetine influences respiration in rats: implications for the treatment of panic disorder. *European Neuropsychopharmacology*, Jan;14(1):29-37, 2004.

III. **Annerbrink K**, Olsson M, Hedner J, Eriksson E. Acute and chronic treatment with serotonin reuptake inhibitors exert opposite effects on respiration in rat: Implications for panic disorder. *Submitted manuscript*.

IV. **Annerbrink K**, Westberg L, Olsson M, Andersch S, Sjödin I, Holm G, Allgulander C, Eriksson E. Panic disorder is associated with the Val308Iso polymorphism in the hypocretin receptor gene. *Submitted manuscript*.

V. **Annerbrink K**, Westberg L, Olsson M, Allgulander C, Andersch S, Sjödin I, Holm G, Eriksson E. Association between the catechol-O-methyltransferase Val158Met polymorphism and panic disorder: a replication. *Submitted manuscript*.

VI. **Annerbrink K**, Westberg L, Nilsson S, Rosmond R, Holm G, Eriksson E. Catechol O-methyltransferase Val158Met polymorphism is associated with abdominal obesity and blood pressure in men. *Metabolism*, May;57(5):708-11, 2008.

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List of abbreviations

BP	Blood pressure
CBT	Cognitive-behavioural therapy
CCHS	Congenital central hypoventilation syndrome
COMT	Catechol-O-methyltransferase
DSM	Diagnostic and statistical manual of mental disorders
HCRT1	Hypocretin receptor-1
HCRT2	Hypocretin receptor-2
mCPP	m-Chlorophenylpiperazine
MV	Minute ventilation
MVP	Mitral valve prolapse
PA	Panic attack
PCPA	Para-chlorophenylalanine
PCR	Polymerase chain reaction
PD	Panic disorder
RR	Respiratory rate
SFA	Suffocation false alarm theory
SNP	Single nucleotide polymorphism
SRI	Serotonin reuptake inhibitor
TD	Tryptophan depletion
TV	Tidal volume

Introduction to panic disorder

Panic disorder (PD) is an anxiety disorder characterized by recurrent, unprovoked panic attacks (PAs) that develop suddenly and peak within minutes. The typical PA can be described as a discrete period of intense physical discomfort accompanied by a fear of losing control, having a heart attack, dying, or going crazy. Respiratory symptoms such as breathlessness, a feeling of being smothered, and hyperventilation, are usually prominent. Other commonly reported symptoms are palpitations, chest pain, sweating, tremor, and dizziness. In addition, PAs are typically accompanied by an urge to flee. The frequency of the PAs vary from several attacks a day to only a few attacks a year, and the severity of each attack can range from limited symptom attacks to full-blown PAs.

Isolated PAs are not uncommon in the general population, so for the criteria of PD according to Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) to be met, the attacks must feature four or more of the symptoms listed in Table 1, and be followed by at least one month of persistent concern about having another attack, worry about the possible implications or consequences of the attacks, or a significant behavioral change related to the attacks (American Psychiatric Association, DSM-IV-TR, 2000). Since PAs can occur in a number of conditions unrelated to PD (i.e. substance abuse, intoxication, hyperthyroidism, and post traumatic stress disorder) differential diagnostic considerations are essential.

PD has an estimated life time prevalence of 3-5% (Grant et al 2006; Kessler et al 1994), and women are 2-3 times more likely to develop PD than men (Eaton et al 1994). The age of onset is typically between late adolescence and early adulthood, and the course is usually chronic (American Psychiatric Association, DSM-IV-TR, 2000). In most long-term studies, a majority of patients report PAs at follow-up, and duration of illness and presence of agoraphobia, rather than severity and frequency of PAs, seem to be negative predictors (Katschnig and Amering 1998). As a chronic disorder, PD can be very debilitating, and patients often report significant social impairment and decreased work ability. In a study by Markowits and co-workers, almost 50% of the patients had been unable to engage in social activities in the last two weeks (Markowitz et al 1989), and Massion and co-workers reported that 65% of patients with PD without agoraphobia were unemployed (Massion et al 1993). In the United States, PD has been reported as the fourth most costly condition of all disorders in terms of decreased work productivity (Kessler et al 2001).

Table 1.

Criteria for Panic Attacks
(American Psychiatric Association, DSM-IV-TR, 2000)
A discrete period of intense fear or discomfort, in which four or more of the following symptoms developed abruptly and reached a peak within 10 minutes:
1. palpitations, pounding heart, or accelerated heart rate
2. sweating
3. trembling or shaking
4. sensations of shortness of breath or smothering
5. feeling of choking
6. chest pain or discomfort
7. nausea or abdominal distress
8. feeling dizzy, unsteady, light-headed, or faint
9. derealisation (feeling of unreality)
10. fear of losing control
11. fear of dying
12. paresthesias (numbness or tingling sensations)
13. chills or hot flushes

Treatment

Serotonin reuptake inhibitors (SRIs) are considered first line treatment for PD. Citalopram, clomipramine, escitalopram, fluoxetine, fluvoxamine, and sertraline have all demonstrated efficacy, and most patients benefit from this treatment (Ballenger et al 1998; Den Boer and Westenberg 1988; Michelson et al 1998; Modigh et al 1992; Pohl et al 1998; Stahl et al 2003; Wade et al 1997). In fact, the effectiveness of the SRIs in PD treatment is remarkable; the vast majority of patients with pure PD hence become panic free within months (Modigh 1987), and controlled clinical trials seldom fail to prove superiority for these drugs over placebo.

This indicates that the effect size of SRIs is in fact superior in PD treatment as compared to their efficacy in the treatment of major depression.

When treatment with SRIs is initiated, an initial increase in anxiety is often experienced by PD patients (Coplan et al 1992). It is thus recommended to start with a low dose that is slowly increased until the effective dose is reached. It usually takes weeks before symptom improvement can be observed, and continuous improvement can be expected for as long as a year after treatment is started.

The monoamine oxidase inhibitors, which also facilitate monoaminergic neurotransmission, are also highly effective in the treatment of PD, but seldom used because of their adverse side effects (Modigh 1987; Tyrer and Shawcross 1988). Benzodiazepines at high dosage are effective but probably less effective than SRIs, and are generally avoided because of their abuse and dependence potential. They can however be of value especially in the early treatment stage before the SRIs have reached their full effect (Andersch et al 1991; Tesar 1990).

Cognitive-behavioural therapy (CBT) in PD includes psychoeducation, anxiety management skills, cognitive reframing, breathing training, and exposure to somatic cues, and has been shown to significantly reduce panic symptoms (Hofmann and Smits 2008; Mitte 2005). Recently, internet-based therapy has also been evaluated as an attractive option with the potential of reaching large number of patients at a reasonable cost (Andersson et al 2005; Kiropoulos et al 2008). There are few randomized studies comparing CBT, SRIs, and placebo, but some studies find the combination of SRIs and CBT to be more effective than either treatment alone (Barlow et al 2000; van Apeldoorn et al 2008).

Biological theories of panic disorder

The pathophysiology underlying PD is not known, but many theories regarding the origin of the disorder have been put forward. Below follows a short description of some of the most influential of these hypotheses.

PD has been discussed in terms of chronic hyperarousal, which could result in a hyperresponsiveness to various anxiety provoking stimuli (Knott et al 1997; Pillay et al 2006; Uchida et al 2008). The underlying reason for this suggested hyperarousal has been discussed in terms of abnormalities in central lactate metabolism (Maddock et al 2008), brain stem structures (Uchida et al 2008), and EEG patterns (Knott et al 1997), and is currently the focus of much interest.

The noradrenergic neurons originating in the locus coeruleus are believed to play a central role in mediating the fight-or-flight response to dangerous or life-threatening events. One theory is that PD results from an augmented synaptic transmission in the locus coeruleus which triggers PAs even in the absence of perceived or actual danger (Kandel 1983). A vicious cycle may occur when either stressful experiences or physiologic stress caused by a medical condition increases locus coeruleus activity, leading to fear-reactions including physiological symptoms such as chest or abdominal pain. These internal physical symptoms may in turn further augment locus coeruleus activity and lead to worsening anxiety (Elam et al 1984; Elam et al 1981; Kandel 1983; Katon and Roy-Byrne 1989; Svensson 1987; Zaubler and Katon 1998).

The neuroanatomical model put forward by Gorman and co-workers hypothesizes that PAs are analogous to a conditioned fear response, and that the attacks are mediated by a fear network centred in the amygdala, hippocampus, medial prefrontal cortex, and hypothalamus. This fear network is suggested to be hyper-sensitive in PD patients most likely due to genetic predispositions, and conditioned to set off either in certain psychological contexts or as a consequence of certain somatic symptoms. (Gorman et al 2000)

Hyperventilation is a common symptom during PAs. The hyperventilation theory suggests that it is the hyperventilation that causes the anxiety attack by decreasing arterial pCO_2 (Ley 1985). This theory has inspired psychological treatment methods, such as breathing training, which have shown positive effects (de Beurs et al 1995; Meuret et al 2004; Roth 2005). Argue against this theory, however, does the fact that it is CO_2-inhalation, rather than monitored hyperventilation, which may cause PAs in PD patients (Garssen et al 1996; Maddock and Carter 1991; Papp et al 1993a; Rapee et al 1992; Zandbergen et al 1990).

An alternative explanation, the suffocation false alarm theory (SFA), was proposed by Donald F. Klein in 1993 (Klein 1993). It postulates the existence of a physiological suffocation alarm system, involving central chemoreceptors that monitor information about potential suffocation. Hyperventilation, panic and an urge to flee are highly adequate responses to threatening hypercarbia or hypoxia, but PAs are believed to occur when the alarm is erroneously activated in susceptible individuals. Contrary to the locus coeruleus and neuroanatomical models described above, a distinction between fear reactions and panic is made by Dr. Klein, who focuses on the fact that PAs are often characterized by marked respiratory symptoms, such as hyperventilation, shortness of breath, and a feeling of being smothered, which are symptoms not generally present during fear. The existence of a suffocation alarm system is illustrated by studies in children with congenital central

hypoventilation syndrome (CCHS, also called Ondine's curse). In this rare condition, the autonomic control of breathing is impaired which leads to sleep apnoeas. If PD is characterized by an abnormally low suffocation alarm threshold, CCHS can be said to have an abnormally high suffocation alarm threshold, and may hence represent the physiological converse of PD. Interestingly, children with CCHS have been found to be significantly less anxious than other chronically ill children investigated (Pine et al 1994).

Serotonin and panic disorder

Regardless of the specific brain regions involved in the pathophysiology of PD, the effectiveness of SRIs suggests that serotonergic neurons are of importance. The influence of brain serotonergic activity on anxiety in PD patients is however complex, as illustrated by the fact that acute administration of SRIs often elicits a paradoxical increase in anxiety, and that the serotonin releasing drugs d-fenfluramine (Garattini et al 1987) and m-chlorophenylpiperazine (mCPP) (Eriksson et al 1999) also provoke anxiety, and even PAs, in PD patients (Charney et al 1987; Hollander et al 1990; Mortimore and Anderson 2000; Targum and Marshall 1989). Why acute facilitation of serotonergic activity elicits anxiety in responsive subjects, whereas long-term treatment with SRIs prevents PAs, is intriguing.

Acute tryptophan depletion (TD) is an effective way to acutely lower brain levels of serotonin by up to 90%, and has been widely used to study serotonergic neurotransmission in human subjects. TD does not exert any major effect on anxiety levels in PD patients, but enhances the panic provoking effects of agents such as yohimbine and CO_2 in PD patients (but not healthy controls) (Goddard et al 1994; Hood et al 2006; Miller et al 2000; Schruers et al 2000). It also augments the ventilatory response to CO_2 in PD patient (Kent et al 1996). Conversely, increasing serotonin availability by subchronic administration of the serotonin precursor L-5-hydroxytryptophan inhibits spontaneous PAs (Kahn et al 1987b); moreover, somewhat unexpectedly, the same compound has also been reported to counteract CO_2-inhalation-induced panic in PD patients also at acute administration (Schruers et al 2000), as does subchronic administration of SRIs (Bertani et al 1997; Perna et al 1997; Shlik et al 1997; van Megen et al 1997).

Also brain-imaging studies have provided support for an involvement of serotonin in PD. There are hence reports suggesting PD to be associated with a reduced density of serotonin transporters as well as 5-HT1A receptors, and that these aberrations are partly restored by effective treatment (Maron et al 2004; Nash et al 2008; Neumeister et al 2004).

Panic disorder and respiration

As briefly discussed above, the possible link between the regulation of respiration and PD has been the subject of extensive studying in the last decades. The interest in this matter stems from the marked respiratory symptoms present during PAs, which distinguish them from fear reactions in general (Klein 1993), and that respiratory stimulants such as CO_2, sodium lactate, pentagastrin, and doxapram have been shown to provoke panic in patients with PD (Abelson and Nesse 1994; Abelson et al 1996; Geraci et al 2002; Gorman et al 1984; Kent et al 2001; Lee et al 1993; Liebowitz et al 1984; McCann et al 1997; Papp et al 1997; Pitts and McClure 1967; Rainey et al 1985; van Megen et al 1994; Woods et al 1986). It has also been shown that PD patients display larger respiratory pattern variability than healthy individuals, both while awake and during sleep, and that this can not solely be explained by more sighing (Abelson et al 2001; Bystritsky and Shapiro 1992; Gorman et al 1988a; Martinez et al 2001; Papp et al 1993a; Papp et al 1993b; Perna et al 1994; Schwartz et al 1996; Stein et al 1995; Wilhelm et al 2001a; Wilhelm et al 2001b).

It has been suggested that PD with respiratory symptoms differ from PD without respiratory symptoms (Klein 1993; Meuret et al 2006; Nardi et al 2008; Onur et al 2007), the respiratory subtypes being more sensitive to panic provocations in the laboratory with substances such as CO_2 and caffeine (Abrams et al 2006; Biber and Alkin 1999; Freire et al 2008; Nardi et al 2007; Nardi et al 2006), and showing respiratory irregularities to a greater extent than the non-respiratory subtype (Beck et al 2000; Bystritsky et al 2000).

Some studies have demonstrated lowered end-tidal CO_2 in patients with PD as compared to healthy controls or patients with other anxiety diagnosis, suggesting chronic hyperventilation (Bass et al 1989; Gorman et al 1984; Hegel and Ferguson 1997; Moynihan and Gevirtz 2001; Papp et al 1997; Rapee 1986; Wilhelm et al 2001b). This has however not been replicated in all studies (Zandbergen et al 1993), and there is evidence to suggest that only PD patients of the respiratory subgroup hyperventilate chronically (Moynihan and Gevirtz 2001), but also evidence that chronic hyperventilation is not diagnostically specific to PD but occurs in non-PD anxiety patients as well (van den Hout et al 1992).

Genetics

Family and twin studies have consistently demonstrated that the etiology of PD is influenced by genetic factors; first-degree relatives of affected probands have a 4-10-fold increase in the

risk of developing PD compared to the general population and twin studies estimate the heritability to up to 50% (Crowe et al 1983; Goldstein et al 1994; Hettema et al 2001; Maier et al 1993; Smoller and Tsuang 1998). The mode of inheritance is most likely multifactorial, with several genes exerting minor effects on the phenotype.

Genetic dissection of complex traits like psychiatric disorders has proven difficult, and positive findings from one study have often not been replicated in independent samples. However, in recent years some specific polymorphisms have been reported to be associated with PD in several independent studies, namely the catechol-O-methyl transferase (COMT) Val158Met polymorphism (Domschke et al 2007), the angiotensin converting enzyme insertion/deletion polymorphism (Bandelow et al 2007; Erhardt et al 2008; Olsson et al 2004c), and the adenosine 2A receptor polymorphism (Deckert et al 1998; Hamilton et al 2004).

Comorbidity

Psychiatric

When concern about the next PA results in avoidant behaviour, the patients may develop agoraphobia. In DSM-IV agoraphobia is defined as anxiety about being in places or situations from which escape might be difficult or embarrassing or in which help might not be available if escape is needed. For the diagnostic criterion to be met, the patient needs to avoid the feared situations (i.e. standing in line, being on a bridge, travelling), endure them with great distress, or require the presence of a companion. Also, the symptoms should not be explained by other conditions such as social or specific phobias (American Psychiatric Association, DSM-IV-TR, 2000).

PD is also highly comorbid with a range of psychiatric disorders apart from agoraphobia, i.e. other anxiety disorders, mood disorders, substance abuse disorders, and somatoform disorders. Depressive disorders, including bipolar disorder, are the conditions most commonly reported to co-occur with PD, followed by other anxiety disorders (Faravelli et al 2004; Jacobi et al 2004; Merikangas et al 1996; Roy-Byrne et al 2000; Weissman et al 1997; Wittchen et al 1998a; Wittchen et al 1998b; Vollrath et al 1990).

Somatic – general

The physiological symptoms of PD can readily be confused with those associated with various medical conditions, both by patients and by health workers. Patients with undiagnosed PD are likely to seek emergency treatment believing that they suffer from a serious medical condition, such as a heart attack. If PD is unrecognized as a possible diagnosis by the medical staff, the patients are likely to receive unnecessarily extensive and costly medical workups for their somatic symptoms, while not receiving adequate treatment for their PD (Zaubler and Katon 1998).

Apart from being confused with various somatic conditions, PD is also closely associated with several somatic illnesses, including vestibular dysfunction (Jacob et al 1996), headache (Marazziti et al 1999), and irritable bowel syndrome (Kaplan et al 1996). The relationship between PD and cardiovascular disorders and respirator disorders, respectively, are discussed below.

Somatic – cardiac

Although much effort is made to explain to the PD patient that their condition is benign, there are in fact reports showing an increase in cardiac morbidity and mortality in PD (Coryell et al 1982; Coryell et al 1986; Smoller et al 2007; Weissman et al 1990; Zaubler and Katon 1996; Zaubler and Katon 1996). A decreased parasympathetic tone, as measured by the heart rate variability between inspiration and expiration, has repeatedly been found in PD patients as compared to controls (Kawachi et al 1995; Klein et al 1995; Yeragani et al 1993; Yeragani et al 1995). Such an imbalance of the autonomic regulation of the heart is a known risk factor for sudden cardiac death (de Bruyne et al 1999; Dekker et al 2000; Tsuji et al 1996), and may thus provide an explanation for the increase in cardiac mortality reported in PD. Effective anti-panic treatment has been suggested to increase heart rate variability (Garakani et al 2008; Tucker et al 1997), and may thus potentially decrease the risk of cardiac mortality. Studies have also found increased QT variability in PD patients (Sullivan et al 2004; Yeragani et al 2002a; Yeragani et al 2000), reflecting an abnormal ventricular repolarisation, which may lead to malignant ventricular arrhythmias and sudden cardiac death (Adamson and Vanoli 2001).

An association between mitral valve prolapse (MVP) and PD has been suggested (Filho et al 2008). It has been speculated that the underlying mechanism is that the tachycardia and high levels of catecholamines associated with PAs may cause desynchronization of the

contractions of the heart and hence to anatomic abnormalities of the mitral valve leaflets, resulting in MVP (Channick et al 1981; Gorman et al 1988b). This hypothesis is supported by findings that MVP associated with PD may be clinically distinct from MVP occurring in the absence of PD (Weissman et al 1987). Researchers have also speculated that the same increase in sympathetic discharge may lead to cardiomyopathy (Gillette et al 1985; Kahn et al 1987a) as well as chronic hypertension which are both found at increased rate in PD patients (Bell et al 1988; Davies et al 1999; Katon 1984; Todd et al 1995; Weissman et al 1990).

Somatic – respiratory

The prevalence of respiratory illnesses, such as asthma and chronic obstructive pulmonary disease, in patients with PD is significantly higher than in patients without psychiatric illness or with other psychiatric disorders (Spinhoven et al 1994; Zandbergen et al 1991). Studies on asthma show that up to 42% of patients experienced panic during asthma attacks and 6.5% to 24% of asthmatic patients meet DSM criteria for PD (Carr 1998; Carr et al 1994; Shavitt et al 1992; Yellowlees et al 1987; Yellowlees et al 1988). When examining patients referred for pulmonary function testing, 17% were found to have PAs and 11% had PD (Pollack et al 1996).

It is debated whether PD leads to respiratory illness, or if respiratory illness leads to PD. The intermittent hypercapnia associated with obstructive pulmonary diseases increases locus coeruleus activity, which could cause panic and hyperventilation (Zandbergen et al 1991). In addition, cognitive processes in anxiety-prone individuals may predispose them to catastrophize somatic sensations associated with respiratory illnesses, and thus make them overly concerned about the consequences of their respiratory symptoms (Carr et al 1994; Porzelius et al 1992). The prevalence of childhood respiratory illnesses among patients with PD is much higher than among patients with other psychiatric disorders, which has led researchers to suggest that these patients may be conditioned to become anxious in response to respiratory symptoms experienced as adults (Zandbergen et al 1991).

Additional background information

The studies presented in this thesis are based on the view discussed above that PD is associated with an aberration in autonomic control of respiration and cardiovascular activity. The aim of papers I-III was to use animal experiments in order to shed further light on the

possibility that serotonin influences the regulation of respiration, and that this influence may be of importance both for the anxiogenic and the anti-panic effects of serotonergic drugs. The aim of paper IV was to examine the possible association between panic disorder and two genes influencing a neuropeptide exerting an established influence on respiration as well as arousal, e.g. orexin. And the aims of paper IV and V was to investigate if a gene that is likely to influence both the autonomic nervous system and the brain, i.e. the COMT gene, is associated with panic disorder (as has previously been suggested), and if it may contribute to the well-established co-morbidity between panic disorder and cardiovascular disease. While I have provided background information regarding panic disorder above, I will, in the following paragraphs, give a brief background to the other specific areas addressed in this thesis, namely i) the regulation of respiration, with special focus on the possible role of serotonin, ii) the neuropeptide orexin, and iii) the COMT Val158Met polymorphism.

Respiratory physiology

The respiratory system aims to maintain proper tissue concentrations of O_2, CO_2 and H^+. In order to achieve this, O_2 is transferred into the blood while, at the same time, CO_2 is removed from the blood. This gas exchange takes place in the alveoli of the lungs where – due to pressure gradients and factors related to chemical affinity – O_2 diffuses from the alveoli into the blood, whereas CO_2 diffuses from the blood into the alveoli.

The main areas involved in ventilatory control are located in the brainstem: 1) the dorsal respiratory group situated in the nucleus tractus solitarius in the dorsal medulla, 2) the ventral respiratory group located in the ventrolateral part of the medulla, and 3) the pontine respiratory group located in the dorsal lateral pons. An intricate interplay between neurons located in these regions, as well as influences from other parts if the brain upon these neurons, will under normal conditions lead to an adequate control of breathing.

Breathing, and thereby the chemical homeostasis of O_2, CO_2, and H^+ in blood and tissues, is constantly controlled by input from the central and peripheral chemoreceptors. These are highly sensitive to changes in gas tension and provide rapid feedback to the brainstem respiratory control system. It is believed that alterations of CO_2 concentration and local tissue H^+ concentration provide the most powerful stimuli for adjustment of ventilation.

The localization of central chemoreceptors is a matter of controversy. Once thought to lie in the surface of the ventral medulla (Loeschcke 1973; Mitchell 1969), they are, according

to more recent evidence. Probably more widely distributed; several brain stem nuclei thus contain chemosensitive neurons that are now candidates for this role, such as the nucleus tractus solitarius and the medullary raphe (Coates et al 1993; Wang et al 1998). The central chemoreceptors monitor H^+ changes in cerebral spinal fluid, which are partly the result of changes in blood CO_2 levels; the blood-brain barrier thus is almost impermeable to H^+, whereas the lipid-soluble CO_2 rapidly passes into the central nervous system and subsequently reacts with water to form carbonic acid that in turn dissociates into H^+ and carbonate.

The peripheral chemoreceptors are located in the carotid bodies, at the bifurcations of the common carotid arteries, and in the aortic body. These receptors are sensitive mainly to changes in O_2, although they respond to changes in CO_2 and H^+ as well. The sensory signals from the peripheral chemoreceptors are transmitted via the vagal and glossopharyngeal nerves into a primary relay station located in the nucleus tractus solitarius of the brain stem.

The number of breaths during a given unit of time is generally referred to as the respiratory rate (RR). The volume of air exchanged via each breath is called tidal volume (TV). The total amount of air exchanged per minute – RR x TV – is termed minute ventilation (MV).

An increase in the partial pressure of CO_2 may be experimentally induced by inhalation of exogenous CO_2. Even small amounts of CO_2 provide a powerful stimulus to the human respiratory control system, increasing both RR and TV and their product MV. Intra-individual respiratory variability – i.e. variations in TV and RR – is a measure assumed to reflect the sensitivity of the mechanisms controlling respiration (Feldman et al 2003).

Serotonin and respiration

Serotonergic nerve terminals are present in many respiratory nuclei, such as the nucleus tractus solitarii, the hypoglossal nucleus, and the preBötzinger complex (Feldman et al 2003; Richerson et al 2005; Richter et al 2003), and researchers have hence investigated the possible connection between serotonin and respiration for decades. However, in what direction serotonin influences baseline respiration, and the response to CO_2, remains a matter of controversy.

When analyzing respiratory data, it is important to take into consideration that factors such as restraint, stress, age, gender, species, anaesthesia, and level of consciousness, may lead to divergences in outcome. Several studies, all performed on anaesthetized or otherwise

pretreated animals, suggest that serotonin is a respiratory *stimulant* (Holtman et al 1987; Lalley 1986; Manzke et al 2003; Martin-Body and Grundy 1985; Millhorn et al 1980; Millhorn et al 1983; Mueller et al 1980; Murakoshi et al 1985; Richerson 2004; Sapru and Krieger 1977; Severson et al 2003; Taylor et al 2004), while other studies, in awake animals, on the contrary point to an *inhibiting* role for serotonin on the regulation of respiration (Annerbrink et al 2003; Bach et al 1993; Mitchell et al 1983; Olson 1987; Struzik et al 2002). Apart from the influence of sleep/wakefulness, another factor that may have contributed to the lack of congruence between studies is the possibility that different serotonergic receptor subtypes may exert different effects on ventilatory regulation.

According to a theory recently launched by Richerson and co-workers, and based mainly on in vitro experiments, serotonergic neurons not only modulate respiration, but actually serve as the central chemoreceptors detecting acidosis and enhanced CO_2-levels (Richerson 2004; Severson et al 2003). This theory is however not undisputed, one counter-argument being that neurons may well detect pH changes in vitro and modulate the central chemoreflex without being chemoreceptors *per se* (Guyenet et al 2008; Mulkey et al 2004). Also, drugs known to radically alter serotonin transmission, such as PCPA, SRIs, and various serotonin receptor agonists and antagonists, do not exert any *dramatic* effects on respiration in humans, which might have been expected if serotonergic neurons were indeed identical to the long-sought central chemoreceptors.

Are genes influencing arousal and/or respiration involved in panic disorder?

In the search for genes influencing the risk of developing PD, genes of importance for respiration and/or arousal are, for reasons discussed above, potential candidates. One pathway related to both respiration and cardiovascular control is the angiotensin system (Atlas 2007; Jennings 1994; Olsson et al 2004b), which has also been suggested to be involved in the pathophysiology underlying PD (Shekhar et al 2006). Supporting the notion that genes of importance for autonomic regulation may be involved in panic disorder , we have previously shown the I allele in the functional angiotensin converting enzyme insertion/deletion polymorphism to be significantly more frequent in male PD patients as compared to controls (Olsson et al 2004c); a finding that has later been replicated by Bandelow and co-workers (Bandelow et al 2007).

A second messenger molecule of importance for respiration (Spyer and Thomas 2000) as well as for arousal (Miller and O´Callaghan 2006) is adenosine. The observation that a polymorphism in the gene encoding the adenosine 2A receptor (Deckert et al 1998; Hamilton et al 2004) appears to be associated with PD hence lends further support for the notion that susceptibility to PD may be related to genes of importance for inter-individual differences with respect to the regulation of respiration and/or arousal.

A third transmitter molecule of possible importance for arousal is neuropeptide S (Jungling et al 2008; Rizzi et al 2008; Vitale et al 2008). Interestingly, a functional polymorphism in the gene encoding this peptide was also recently associated with PD in male patients (Okamura et al 2007). This finding however still awaits replication.

Orexin is another neuropeptide that regulates arousal (Adamantidis and de Lecea 2008), and that is also involved in the regulation of respiration (Williams and Burdakov 2008), hence making it an intriguing candidate in the search for PD-related genes. However, as yet no studies regarding the possible role of orexin-related genes in PD have been published (see below).

Serotonin-related genes, such as the serotonin transporter promoter polymorphism (5-HTTLPR), have been thoroughly investigated in PD, but so far with negative or conflicting results (Blaya et al 2007). We are in the process of genotyping a number of serotonin-related genes in our PD population, but the results thus far are disappointing.

Orexin

Orexins (also called hypocretins) are excitatory neuropeptide hormones. While neurons containing orexin originate almost exclusively from the lateral hypothalamus perifornical area and the dorsomedial hypothalamus (de Lecea et al 1998; Kuwaki 2008; Sakurai et al 1998), orexin containing nerve terminals are widely distributed in the hypothalamus, thalamus, cerebral cortex, circumventricular organs, brain stem, and spinal cord, where they influence a wide range of physiologic and behavioral processes related to food-intake, wakefulness, and metabolism (de Lecea et al 1998; Elias et al 1998; Nambu et al 1999; Sakurai et al 1998). One of the key roles for orexin is to regulate sleep and wakefulness by activating monoaminergic and cholinergic neurons in the hypothalamus and brain stem which maintain wake-periods; the fact that orexin deficiency causes narcolepsy underlines its importance in this context (Sakurai 2007). A key role for orexins in the arousal response to fear-related stimuli has also been suggested; prepro-orexin-knockout mice show diminished locomotor and cardiovascular

response in the resident-intruder paradigm designed to induce emotional stress (Kayaba et al 2003), and orexin/ataxin 3 transgenic mice with ablated orexin neurons display diminished cardiovascular response to air-jet stress paradigm (Zhang et al 2006a).

Orexin is also believed to play a role in respiratory regulation, especially in awake states (Kuwaki 2008). Axons of orexin-containing neurons project to respiration-related sites, such as the nucleus tractus solitarius, pre-Bötzinger complex, and the hypoglossal, raphe, retrotrapezoid, and phrenic nuclei (Berthoud et al 2005; Fung et al 2001; Peyron et al 1998; Young et al 2005). Intracerebroventricular administration of orexin promotes respiration (Zhang et al 2005) and orexin-deficient mice exposed to stressors increase their respiration less as compared to control mice (Kayaba et al 2003; Zhang et al 2006b). Also, prepro-orexin knockout mice were found to have higher RR and lower TV than wild type mice, and their response to CO_2 is decreased. The attenuated response to CO_2 can be partly restored by supplementing orexin; moreover, the orexin antagonist SB-334867 decreases the MV response to CO_2 when administered to wild type mice (Zhang et al 2006b).

Orexin has recently been suggested to be a key substance in PD since orexin cells in the dorsomedial hypothalamus express c-Fos in panic-prone rats after lactate infusion, but not in controls (Johnson et al 2008), and since systemic silencing of prepro-orexin gene expression using RNA interference methods blocks lactate-induced increases in heart rate and blood pressure (BP), and attenuates expression of anxiety-like behaviour on the social interaction test (Truitt et al 2007).

The gene encoding catechol-O-methyl transferase

COMT is a catabolic enzyme located in postsynaptic neurons that deactivates the catecholamine neurotransmitters dopamine, noradrenaline and adrenaline, as well as other substances with a catechol structure (such as catecholestrogens). The COMT gene contains a functional polymorphism, the COMT Val158Met polymorphism that results in an amino acid substitution of methionine (Met) for valine (Val) at codon 158. The Met allele is thermolabile and has one-fourth of the enzymatic activity of the Val allele (Lachman et al 1996; Lotta et al 1995). Since COMT metabolizes important neurotransmitters, the Val158Met polymorphism has been extensively studied in association studies. The results have been conflicting, but several studies suggest this polymorphism to be linked with schizophrenia (Lewandowski 2007) and cognitive function (Tunbridge et al 2006). In addition, several studies suggest that the Val allele is more common in female Caucasian PD patients, as compared to controls

(Domschke et al 2004; Hamilton et al 2002; Rothe et al 2006). Specifically, the Val allele appears to be more common in female Caucasian PD patients, as compared to controls (Domschke et al 2007).

As described above, PD is associated with increased cardiovascular mortality. Since the COMT enzyme plays an important role in inactivating catecholamines, the COMT Val158Met polymorphism could tentatively provide a link between PD and cardiovascular disorders. To what extent the COMT Val158Met polymorphism is associated with cardiovascular disease however remains to be established.

Papers I-VI: Aims, Results, and Discussion

PAPER I: Does serotonin depletion obtained by means of para-chlorophenylalanine administration alter baseline ventilation, CO2 response, and respiratory variability in rat?

To further explore the involvement of serotonin in respiratory regulation, we examined the effect of the serotonin synthesis inhibitor PCPA, at a dose previously shown to cause a marked reduction in brain serotonin levels in rat (Eden et al 1979), on baseline respiratory patterns and on CO_2 responsiveness in freely moving male Wistar rats.

PCPA induced an overall hyperventilation at baseline due to an increase in TV accompanied by a decrease in RR. After CO_2 exposure, there was no difference between controls and PCPA-treated rats regarding MV, although PCPA-treated animals maintained a higher TV and lower RR. The finding that PCPA induced hyperventilation without affecting CO_2 responsiveness is a replication of earlier findings in rats (McCrimmon 1995), and is well in line with the finding by Struzik and co-workers showing that lowering serotonin levels in man by means of tryptophan depletion elevates baseline respiration without affecting responsiveness to CO_2 (Struzik et al 2002).

Of the serotonergic compounds investigated in this thesis (se also Paper II-III), only PCPA actually affected MV. It is possible that, in the doses administered, only PCPA caused a dramatic enough alteration in serotonin levels for a serotonergic effect on MV to be apparent. However, not even PCPA affected the CO2 response. The results thus support a modulatory role for serotonin in respiratory regulation, rather than a role for serotonergic neurons as chemoreceptors (as recently suggested) (Richerson 2004).

In line with the notion that serotonergic neurons may serve as chemoreceptors, many studies have suggested serotonin to be a respiratory stimulant (Holtman et al 1987; Lalley 1986; Manzke et al 2003; Martin-Body and Grundy 1985; Millhorn et al 1980; Millhorn et al 1983; Mueller et al 1980; Murakoshi et al 1985; Richerson 2004; Sapru and Krieger 1977; Severson et al 2003; Taylor et al 2004), which is not consistent with our findings. This discrepancy may be due to the fact that the rats in our study were awake and unrestrained, whereas the animals in the studies cited above all had been anesthetized or otherwise pretreated.

Respiratory variability, i.e. the intra-individual variability in TV and RR, is enhanced in patients with PD (Abelson et al 2001; Martinez et al 2001; Papp et al 1997; Stein et al 1995;

Yeragani et al 2002b). We found that TV and MV variability, but not RR variability, were higher in PCPA-treated rats as compared to control rats both at baseline and during CO_2exposure. The observation that serotonin depletion enhances respiratory variability in rat, in conjunction with the study by Yeragani and co-workers showing that serotonin reuptake inhibition may decrease respiratory variability in PD patients (Yeragani et al 2004), makes it tempting to speculate that an aberration in brain serotonergic transmission may be a mechanism of importance for the increase in respiratory variability associated with PD. If so, a hypothetical reason for the beneficial effect of the SRIs in PD may be an ability of these compounds to stabilize respiration.

PAPER II. Does serotonin reuptake inhibition alter baseline respiration and CO_2 response?

Successful antipanic treatment antagonizes not only spontaneous but also panicogen-induced anxiety attacks (Bocola et al 1998; Gorman et al 1997; Perna et al 2001; Pohl et al 1998; Pols et al 1996). If the panic response induced by CO_2 and lactate is due to cognitive misinterpretation of non-specific somatic stimuli (Goldberg 2001), and/or to a hyperreactive fear network (Gorman et al 2001; Sinha et al 2000), the counteracting influence of SRIs on the panic response could be explained by an influence of these drugs on cognitive function and/or on fear generating circuits. On the other hand, if CO_2- and lactate-induced PAs are due to the effects of these compounds on respiration, an explanation for the beneficial effect of SRIs could be related to the ability of serotonin to modulate respiration. It has been suggested that CO_2- and lactate-induced panic is specifically related to an activation of chemoreceptors (Klein 1993); a possible mechanism of action for the antipanic effect of SRIs could thus tentatively be to reduce chemoreceptor hyperresponsiveness.

The finding that SRIs reduce the ventilatory response to CO_2 in PD patients (Bocola et al 1998) may be regarded as support for the assumption that they influence chemoreceptor responsiveness. However, since the ventilatory response to CO_2 in subjects experiencing panic could be due to heightened anxiety, the attenuated hyperventilation seen after SRI treatment could also be secondary to the antipanic effect rather than due to a direct influence on neuronal circuits involved in the regulation of respiration.

In order to examine the possible effect of antipanic treatment on chemoreceptor responsiveness, we studied the effect of the SRI paroxetine on baseline respiration and on CO_2 response in freely moving Wistar rats. The most consistent finding of these experiments

was that paroxetine caused an increase in baseline RR both after 5 and 15 weeks of treatment, without significantly affecting TV or MV. The increase in RR following CO_2 exposure was reduced after 15, but not 5, weeks of paroxetine treatment.

In conclusion, these findings do not suggest that paroxetine affects chemoreceptor responsiveness since no overall change in MV was apparent during the experiment. However, the results do suggest that chronic treatment with paroxetine modulates respiratory pattern, both at baseline and – after long term treatment – during CO_2 exposure, so that RR is enhanced and TV reduced.

The lag phase with respect to the effect of paroxetine on CO_2 response does not argue against the possibility that this effect may contribute to the antipanic effect of these drugs in man. When used to treat PD, the SRIs thus display a considerable delay with respect to onset of action, and continuous improvement is observed months after treatment has been initiated (Davidson 1998; Modigh et al 1992).

PAPER III. a) Is the respiratory effect of acute SRI administration opposite to that of chronic treatment? b) Do other serotonergic drugs also affect respiration?

The aim of the present study was to compare the respiratory effect of acute SRI administration with that of chronic treatment, the *à priori* hypothesis being that acute administration would exert the opposite effect of chronic treatment – i.e. that it would *reduce* RR – just as acute SRI administration exerts the opposite effect to that of chronic treatment on anxiety in PD patients.

In Paper II we showed that administration of the SRI paroxetine for 5 or 15 weeks increases RR in awake, unrestrained freely moving rats (Olsson et al 2004a). This finding was confirmed in the present study; a significant increase in RR was thus observed after 23 days of fluoxetine administration and onwards. Analysis of fluoxetine and norfluoxetine showed a gradual increase in serum levels as measured 24 h after the latest drug injection. This increase, however, can not by itself explain the increase in RR, since even higher serum levels were observed 1 h after drug injection without a reduction in RR to be at hand.

When SRIs were administered acutely, we instead observed a dose-dependent *decrease* in RR. Acute administration of SRIs increases synaptic levels of serotonin by blocking the reuptake inhibitor (Stahl 1998) but, at the same time, also silences serotonergic nerve cell activity due to autoreceptor (5HT1A) activation (Aghajanian et al 1970; Blier and de Montigny 1985). If serotonergic neurons stimulate respiration by chemoreceptor activation (see above), an inhibition of serotonergic cell firing may indeed lead to reduced RR. The

observed effect on RR could thus be explained either in terms of enhanced extracellular concentrations of serotonin, or by a feed-back inhibition of serotonergic neurons.

To address the latter possibility, we administered the 5-HT1A agonist 8-OH-DPAT at doses known to effectively reduce the firing rate in serotonergic neurons without influencing postsynaptic 5-HT1A receptors (Blier and de Montigny 1990; Forster et al 1995; Sharp et al 1989). Since there was no observable effect on respiration after 8-OH-DPAT administration, no support was obtained for the theory that the effect of SRIs is secondary to 5-HT1A-mediated inhibition of serotonergic neurons. Another study in awake freely moving guinea pigs showed an increase in RR after 8-OH-DPAT administration (Stone et al 1997). Although this observation differs from our negative finding, it also argues against the notion that inhibition of serotonergic cell firing reduces RR. Notably, in humans, 5HT1A agonists neither reduce panic attacks nor elicit them (Sheehan et al 1993; van Vliet et al 1996) which is in line with the lack of effect of low-dose 8-OH-DPAT administration on respiration.

To explore the alternative explanation to the reduction in RR seen after acute SRI administration, i.e. that it is secondary to enhanced synaptic serotonin levels, two compounds known to effectively increase extracellular levels of serotonin, mCPP and d-fenfluramine (Eriksson et al 1999; Laferrere and Wurtman 1989), were administered. Both these compounds caused a robust decrease in RR, thus supporting the notion that the effect of acute SRI administration on RR is secondary to enhanced serotonin levels in the synapse.

The 5HT1A antagonist WAY-100635 enhances the firing of some but not all serotonergic neurons in awake animals (Fornal et al 1996; Kasamo et al 2001; Mlinar et al 2005). The observation that WAY-100635 elicited the same effect as acute administration of fluoxetine, paroxetine, mCPP, and d-fenfluramine, *i.e.* a reduction in RR, thus further supports the idea that serotonin exerts an inhibitory influence on this parameter.

When WAY-100635 is co-administered with an SRI, the former drug may counteract the inhibitory influence of the latter on serotonergic cell firing, and hence potentiate the stimulatory influence on extracellular levels of serotonin (Arborelius et al 1996; Hjorth 1993; Invernizzi et al 1996). The finding that WAY-100635 plus fluoxetine did not reduce RR significantly more than did either drug alone (although the effect of the combination was numerically larger) suggests that RR-modulating serotonergic neurons are not inhibited by SRIs. This may also explain why 8-OH-DPAT had no observable effect on respiration.

The present observation, that the effect of acute administration of an SRI on RR in rat is opposite to that of subchronic treatment adds to an already existing body of data suggesting that the effect of subchronic treatment with SRIs on serotonergic output, at least in some

neuronal circuits, is opposite in direction to that observed after acute administration (Stahl 1998). SRIs are not only devoid of antipanic effects during the first days of PD treatment, but initially often aggravate anxiety (Nutt and Glue 1989; Pohl et al 1988), and drugs causing a marked and immediate increase in synaptic serotonin concentrations, such as fenfluramine and mCPP, do not exert acute anti-anxiety effects, but, on the contrary, may elicit anxiety in patients with panic disorder (Charney et al 1987; Mortimore and Anderson 2000). Recently, acute SRI administration to man has been found to enhance amygdala reactivity (Bigos et al 2008) whilst subchronic treatment reduces it (Harmer et al 2006).

As the results of Paper II, those of paper III do not suggest that serotonergic drugs in the doses used induce either a hyper- or hypoventilation, but rather modulates the respiratory pattern. We hypothesize that respiratory irregularities caused by serotonergic imbalance may be of central importance for both anxiety symptoms and respiratory irregularities observed in patients with PD, and that the effects of serotonergic drugs on anxiety in PD patients may in fact be secondary to effects on respiration. In line with this, the effects of fenfluramine, mCPP, and acute administration of SRIs with respect to RR in rat were opposite to that seen after subchronic treatment, just as all the former treatment enhance anxiety whereas the latter prevents panic attacks.

The experiments presented in papers I-III in this thesis were all performed on rats, an animal often used to study respiration. In an ongoing clinical study we are exploring the effects of acute and subchronic SRI administration on respiratory parameters in patients with panic disorder and depression, respectively, the hypothesis being that acute administration will reduce RR, and that this effect will be correlated with an initial increase in anxiety in PD patients. In contrast, we predict long-term treatment to be associated with an increase in RR that will be accompanied by a prevention of panic attacks. In addition, a possible influence on respiratory variability as well as on CO_2 responsiveness will be assessed.

PAPER IV. Is panic disorder associated with the polymorphisms HCRTR1 Ile408Val and HCRTR2 G1246A in orexin receptors 1 and 2?

Since PD has been suggested to be due to an aberration in the control of respiration, the neuropeptide orexin (hypocretin), – which is believed to play a key role in respiratory regulation (Creveling 2003; Kayaba et al 2003; Kuwaki 2008; Zhang et al 2005; Zhang et al 2006b) – is a transmitter of possible importance for PD pathophysiology. In this vein, studies showing orexin cells in the dorsomedial hypothalamus to express c-Fos after lactate infusion

in panic-prone rats but not in controls, and silencing of the prepro-orexin gene expression to impede the increase in heart rate, BP, and anxiety-like behaviour seen after lactate-infusion, recently prompted the suggestion that orexin may indeed be of importance for the development of PD (Truitt et al 2007). Similarly, the suggested role for orexin in mediating arousal (Sakurai et al 2005; Winsky-Sommerer et al 2004; Yoshida et al 2006) is intriguing in this context, since PD has been proposed to be associated with a state of chronic hyperarousal (Knott et al 1997; Pillay et al 2006; Uchida et al 2008).

To explore the possible importance of orexin for PD in humans – which to our knowledge never has been done before – we investigated the possible association between PD and two polymorphisms in the orexin receptors: the Ile408Val polymorphism in the gene encoding the hypocretin receptor 1 (HCRTR1) and the Val308Iso (G1246A) polymorphism in the gene encoding the hypocretin receptor 2 (HCRTR2). We chose these two polymorphisms because they have previously been associated with biological traits; the HCRTR2 Val308Iso polymorphism has thus been associated with cluster headache (Rainero et al 2007; Schurks et al 2006) and the HCRTR1 Ile408Val polymorphism to polydipsia in schizophrenic patients (Fukunaka et al 2007; Meerabux et al 2005).

Whereas no association between the HCRTR1 Ile408Val polymorphism and PD was found, the A-allele of the HCRTR2 Val308Iso polymorphism was significantly more frequent in patients than in controls. After dividing the populations according to gender, this association was seen in female patients only. Gender-specific effects of various polymorphisms are not unusual; however the small sample size, and therefore lack of power, in the male patient population, must also be considered when interpreting this difference.

The HCRTR2 gene is located on chromosome 6p12.1, consists of 7 exons (100 kbp), and is expressed exclusively in the brain (Sakurai et al 1998). The possible functional significance of the valine to isoleucine amino acid substitution at position 308 is as yet unknown, but it has been suggested to interfere with the dimerization process of the receptor (Rainero et al 2007).

Since this is a novel finding, it needs to be replicated in independent samples. We however suggest that future research regarding the role of orexin in PD may prove fruitful.

PAPER V. Is the association between the COMT Val158Met polymorphism and panic disorder possible to replicate?

COMT is a ubiquitous enzyme of importance for the degradation of both catecholamines and estrogens (Creveling 2003). The coding region of the COMT gene comprises a single nucleotide polymorphism (SNP) resulting in a Val to Met substitution (Val158Met). This SNP has been shown to influence the activity and thermal stability of the enzyme *in vitro* (Lachman et al 1996; Lotta et al 1995), the Val allele resulting in up to four times the enzymatic activity of the Met allele at body temperature (Chen et al 2004; Lachman et al 1996). Catecholamines have been attributed importance for the generation of panic attacks (Kandel 1983), and they also mediate the sympathetic input on the heart; variations in COMT activity may thus be linked both to the psychological aspects of PD and to some of the somatic consequences, such as high BP (Wilkinson et al 1998) and enhanced mortality in cardiovascular disease (Coryell et al 1982; Coryell et al 1986; Smoller et al 2007; Weissman et al 1990; Zaubler and Katon 1998; Zaubler and Katon 1996).

An association between the Val158Met polymorphism and PD does indeed get support from several independent studies suggesting the Val allele to be more common in Caucasian PD patients than in controls; after splitting for gender, these associations were however found only in the female subgroups. (Domschke et al 2007). The association between this polymorphism and PD being one of the more promising findings from psychiatric association studies, we deemed it important to address if it could be replicated in our group of PD patients as well. We indeed found the Val158 allele to be significantly more frequent in PD patients than controls; moreover, when splitting for gender, the association was significant in both male and female subgroups. Since all PD populations investigated to date are relatively small, it is possible that the lack of association in male patients in earlier studies have been due to low power.

Interestingly, in Asian samples an association opposite to that observed in Caucasian samples has been found; in Asian samples, it is thus the Met allele of the Val158Met polymorphism, rather than the Val allele, that appears to be associated with PD (Woo et al 2004; Woo et al 2002). Since any functional polymorphism involved in an important biological pathway may be expected to elicit numerous adaptive mechanisms, its net effect on the phenotype is likely to be dependent on the presence of other polymorphisms in genes encoding other proteins in the same pathway. This may thus explain why one allele may predispose to a certain trait in subjects from one part of the world whereas the other allele of

the same polymorphism may be associated to the same trait in other regions. Also, the possibility that a polymorphism that is in linkage disequilibrium with the investigated polymorphism in one part of the world, but less so in another region, is of functional importance, and responsible for the observed association, should not be disregarded.

Several studies also suggest a role for the COMT Val158Met polymorphism and other anxiety-related traits in women. In these studies, it is however usually the Met allele that appears to be associated with traits such as harm avoidance (Enoch et al 2003), episodic anxiety (Olsson et al 2005), low extraversion, high neuroticism (Stein et al 2005), and heightened reactivity in corticolimbic circuits (Drabant et al 2006); in contrast, Hettema and co-workers found an association between the Val allele on the one hand and neuroticism and anxiety disorders on the other (Hettema et al 2008). The Val158Met polymorphism has also been studied in relation to processing of emotional stimuli in amygdala and prefrontal cortex in PD patients and healthy probands with positive although conflicting results; PD patients carrying the Val allele reacted more to faces expressing negative emotions (Domschke et al 2008) whereas healthy subjects carrying the Met allele were found to react more to unpleasant stimuli (Smolka et al 2005).

Of possible relevance in this context is also the association between the COMT Val158Met polymorphism and cognition. Dopamine levels in the prefrontal cortex are critical for modulating cognitive function, and hence the COMT Val158Met polymorphism has been thoroughly investigated with regard to cognitive tasks. In these studies, this polymorphism has been relatively consistently shown to modulate performance on tasks related to prefrontal cortex activation (Bilder et al 2002; de Frias et al 2005; Diamond et al 2004; Egan et al 2001; Goldberg et al 2003; Joober et al 2002; Malhotra et al 2002; Mattay et al 2003); in short, the Met allele seems to be associated with better performance on tasks involving working memory and executive functioning, but also associated with impaired emotional processing (Tunbridge et al 2006).

The tonic/phasic model of dopamine system regulation has been used to explain the complex relationship between cognitive function and the COMT Val158Met polymorphism (Bilder et al 2004; de Frias et al 2008; Wilkerson and Levin 1999). In this model, consideration is given to what type of dopaminergic activation that is required to perform a certain task. The tonic activity is suggested to be characterized by a constant, slow firing of dopamine neurons in the prefrontal cortex, and of assumed importance for sustained attention. The phasic activity, on the other hand, is characterized by transient, high-amplitude dopaminergic activity, and critical for updating and gating new information. While the Met

allele is assumed to augment the tonic component leading to increased stability but decreased flexibility, the Val allele is assumed to promote the phasic component. This may be relevant also to the relationship between Val158Met on the one hand and PD and other anxiety disorders on the other; while Val carriers could be assumed to filter sensations generated by increased autonomic arousal less effectively, Met carriers may be characterized by a lack of flexibility that makes them more likely to brood and get stuck in negative thought-spirals.

In this context, it should also be stressed that broadly defined anxiety is not to be regarded as pathological, but, on the contrary, adds useful diversity needed for long-time survival of the species. It is probably rarely the case that one allele variant is always beneficial, whereas the other is always detrimental; rather, different variants probably result in phenotypes that may all be of benefit depending on the requirements.

In conclusion, our results, together with previous studies, strongly suggest that the COMT Val158Met polymorphism is of importance for the development of PD. We suggest this to be one of the more robust findings obtained in psychiatric association studies so far.

In conclusion, our results, together with previous association studies, strongly suggest that the COMT Val158Met polymorphism is of importance for the development of PD, and further studies regarding the role of catecholamines and autonomic control in PD patients are highly warranted.

PAPER VI. Is the COMT Val158Met polymorphism associated with cardiovascular risk factors?

The COMT enzyme degrades catecholamines and estrogens (Creveling 2003) – both of which are of known importance for cardiovascular risk factors such as obesity and hypertension (Esler 1993; Halford et al 2004; Louet et al 2004; Muller et al 2003; Tchernof and Despres 2000) – and the COMT gene has previously been associated with hypertension (Hagen et al 2007; Kamide et al 2007). This gene being associated both with PD and with cardiovascular disease might hence be one contributing factor to the co-morbidity between the two.

To further explore this possibility, we investigated the possible association between the COMT Val158Met polymorphism on the one hand, and BP and anthropometry on the other, in 240 middle-aged Swedish men, all 51 years old, who were recruited by means of the population register and free from antihypertensive medication.

Subjects with two copies of the low-activity Met158 allele had significantly higher waist-hip-ratio, sagittal diameter, systolic BP, diastolic BP, and heart rate than subjects with

two copies of the high-activity Val158 allele, heterozygous subjects displaying values in between. This is intuitively attractive since the Met allele results in slower degradation of catecholamines, which could be expected to raise BP and heart rate. However, the only previous study examining the effect of the COMT Val158Met polymorphism and BP found an association between systolic BP and the low-activity Val158 allele in a large Norwegian population (Hagen et al 2007).

Our *á priori* hypothesis was that the COMT Val158Met polymorphism could provide a link between PD and the elevated BP often observed in PD patients (Bell et al 1988; Davies et al 1999; Katon 1984; Todd et al 1995; Weissman et al 1990) as well as with the enhanced mortality in cardiovascular disease associated with PD. However, since we found the Val158 allele to be associated with PD, but carriers of the Met158 variant to have higher BP and higher WHR, no support for this theory was gained.

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Summary

- Serotonin depletion with PCPA induced hyperventilation in freely moving rats due to an increase in TV, but did not affect CO_2 response. This observation – that is in line with some previous studies – is of importance, since it refutes the theory based on *in vitro* studies, and studies using anaesthetized animals, that serotonin is crucial for CO_2 responsiveness and that it exerts mainly a *stimulatory* influence of respiration. PCPA also increased respiratory variability, suggesting that the respiratory abnormalities observed in patients with PD may be due to alterations in brain serotonergic neurotransmission.

- Acute treatment with the SRIs paroxetine and fluoxetine decreased RR, as did acute treatment with the serotonin releasing drugs d-fenfluramine and m-CPP and the 5-HT1A antagonist WAY-100635. Due to a corresponding increase in TV, there were however usually no significant effects on MV. The results suggest that acutely increasing serotonin levels in the synapse reduces rather than increases RR, and hence reinforce the conclusion that serotonin does not exert any clear-cut stimulatory influence on respiration in awake animals.

- Chronic treatment with the SRIs fluoxetine and paroxetine for at least three weeks exerted an effect on RR opposite to that obtained by acute administration of SRIs (as well as of serotonin releasers), i.e. it *increased* RR. As with acute treatment, no marked effect on MV was observed – again suggesting that serotonin plays a modulatory role on respiratory pattern, rather than exerting a clear-cut stimulatory or inhibitory influence. The opposite effects of acute and chronic SRI administration, respectively, may be the result of adaptive processes in certain neuronal circuits obtained by long-term but not acute SRI exposure. In line with our results, acute administration of SRIs, fenfluramine and mCPP enhances anxiety in PD patients, whereas long-term administration with SRIs prevents panic attacks. Further studies are required to establish if the effects of these drugs on respiration are of direct importance for their effects on anxiety in PD patients, or if reversal of the functional effects of acute SRI to its opposite upon long-term administration is a general

phenomenon that occurs independently in both respiration-modulating and anxiety-modulating serotonergic pathways.

- A polymorphism in the orexin-receptor 2, HCRTR2 G1246A, was significantly associated with panic disorder in female patients only. This is the first report providing direct support for an involvement of the respiration- and arousal-modulating peptide orexin in panic disorder, and the result should hence be interpreted with caution until replicated. Our observation is however in excellent agreement with preclinical data suggesting orexin to play a pivotal role in an animal model of PD.

- The Val allele in the COMT Val158Met polymorphism was found to be significantly more frequent in PD patients than controls. This is a replication of earlier findings in Caucasian samples, although we found the association in both male and female subgroups whereas earlier studies have demonstrated the effect in female patients only. We suggest that the association between the Val allele of the COMT Val158Met polymorphism and panic disorder may by now be regarded as one of the few findings from psychiatric association studies that could be regarded as definitely confirmed.

- The COMT Val158Met polymorphism was also associated with cardiovascular risk factors in our sample of healthy middle-aged men. The low activity Met allele was however associated with significantly higher WHR, sagittal diameter, systolic blood pressure, diastolic blood pressure, and heart rate; this study hence provided no support for the theory that the COMT gene may contribute to the association between PD and cardiovascular mortality.

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Appendix: Material and methods

Animal studies (Paper I-III)

Ethics

The studies have has been carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the NIH (NIH publication No. 85-23, revised 1985), and were approved by the Ethics Committee for Animal Experiments, University of Gothenburg, Sweden.

Animals

Male Wistar rats were used. Before the experiments, the rats were housed under controlled conditions: temperature 21-22ºC, humidity 55-65%. Food and water were available ad libitum at all times except during the experiments.

Respiratory measurement

All experiments took place in a silent room with lights on and an observer placed approximately 1 m from the rat. Two different equipments were used to measure respiration in freely moving rats: In Paper I-III, the plethysmograph used to measure ventilation was built in our laboratory, made of Plexiglas and cylindrical in shape (height 235 mm, diameter 290 mm, volume 15.5 l). A sensor membrane, responding to pressure differences in the plethysmograph caused by the animal breathing, was connected to a Macintosh computer (software: MacLab) via a transducer.). In Paper III, the Unrestrained Whole Body Plethysmograph from Buxco Research Systems (Wilmington, NC, USA) was also used. The animals were able to move freely inside the plethysmograph at all times.

Before the day of the acute experiment, the animals were habituated to the plethysmograph on three different days (15 min + 15 min + 15 min). Before recording started on the day of the experiment, the animals were rated by means of gross observation with respect to motor activity. Animals that were not still after having spent 10-15 minutes in the plethysmograph were disqualified. The rater was blind as to whether the rat was given saline

or active substance. RR (breaths per min=BPM), TV (ml/breath), and MV (ml/min) were registered during two minutes.

Gas exposure (Paper I-II)

The gas used was either 100% CO_2 or a control gas consisting of 20% O_2 and 80% N_2. The gas was administered through a valve in the plethysmograph, with a constant inflow rate of approximately 3 litres per minute, regulated by a BS300 regulator and a Dynamal inflow-rate meter (Air Liquide, Gothenburg, Sweden). Duration of gas administration was 10-35 seconds depending on the CO_2 concentration desired. Another valve was used for pressure monitoring. A 2001 VTCM gas meter (Comfort-Control, Uppsala, Sweden) was used to measure the level of CO_2 as well as the temperature within the plethysmograph.

Analysis of serum paroxetine and fluoxetine (Paper II and III)

Mixed arteriovenous trunk blood was collected for analysis after decapitation of the rats. The blood was allowed to clot for 20–30 min in the collecting test tube at room temperature and then centrifuged at 2500 g for 10 min. Supernatant serum was then transferred into a new test tube, frozen, and kept stored at −70 °C until analysis was performed.

Serum concentrations of paroxetine were assessed using high-performance liquid chromatography connected to a UV-detector working at an excitation/emission wavelength of 210 nm at the Department of Psychiatry, Linköping University Hospital.

Serum concentrations of fluoxetine and norfluoxetine were assessed using high-performance liquid chromatography followed by liquid chromatography tandem mass spectrometry at the Division of Clinical Chemistry and Pharmacology, Lund University Hospital.

Statistics

Between-group and in-group differences were evaluated statistically using ANOVA followed by Fisher's PLSD test (Paper I-III), unpaired t-test (Paper I-III), or paired t-test (Paper I and III). In addition, two way ANOVAs were performed in Paper I. All values are expressed as mean (±SD) and $p \leq 0.05$ was considered statistically significant.

Genetic studies (Paper IV-VI)

Ethics

All participants provided written informed consent. The study protocol was approved by the Ethics committees at the University of Gothenburg and the Umeå University.

Subjects

The subjects in Paper VI were drafted from the general population of Göteborg, Sweden, and consisted of 240 men born in 1944 who were recruited for a study of obesity, anthropometrics, and cardiovascular risk factors (Rosmond et al 1998). These subjects were also used as controls in Paper IV and V, as were 269 women born in 1956 and recruited for the same purpose (Rosmond and Bjorntorp 1998).

The patients with PD in Paper IV and V were recruited from the Göteborg Anxiety Syndrome Society (Ångestsyndromsällskapet, Göteborg, Sweden), from the private psychiatric practices of Dr Sven Andersch, Gothenburg, and Dr Christer Allgulander, Stockholm, and among subjects who had participated in controlled drug trials.

Molecular genetics

Venous blood was collected from each subject, and genomic deoxyribonucleic acid was isolated using the QIAamp DNA blood Mini Kit (Qiagen, Chatsworth, CA).

Pyrosequencing® (Paper IV-IV)

Pyrosequencing (Nordfors et al 2002) is a method of DNA sequencing using the polymerase transcription process itself. To initiate incorporation of nucleotides at the desired location the DNA polymerase uses a short sequence primer, which hybridizes close to the investigated polymorphism. The nucleotides are added in a pre-programmed order, and when a nucleotide is incorporated the release of pyrophosphate starts a luciferase-catalyzed enzymatic reaction generating visible light which can be detected by a charge coupled device camera and seen as a peak in a pyrogram. The Assay Design Software, Biotage Version 1.0.6 was used.

Sequenom® (Paper IV and V)

Sequenom is a high-throughput SNP analysis tool based on multiplex polymerase chain reaction (PCR) with subsequent single base primer extension, followed by an analysis with MALDI-TOF-MS (van den Boom and Ehrich 2007). One extension primer per SNP is added to the PCR products together with the nucleotides. The extension primers anneal to their specific PCR product, the base before the SNP and the following extension is depending on the allele. The extension product will be elongated either with one or two bases and their different masses are separated in the mass spectrometer, rendering a spectrogram with the genotypes of all SNPs in the plex. TyperAnalyzerFS © software Version 1.0.1.46 was used to assess the results.

Genotyping

The COMT Val158Met polymorphism (SNP ID: rs4680) was analysed using Pyrosequencing (Paper VI) or Sequenom (Paper V). The HCRTR2 G1246A polymorphism (SNP ID: rs2653349) was assessed using Sequenom and, for samples for which the Sequenom analysis failed, with Pyrosequencing. The HCRTR1 Ile408Val polymorphism (SNP ID: rs2271933) was analyzed using Pyrosequencing. All primers used in the analyses are listed in Table 2.

Table 2a. Primers used in Pyrosequencing

Gene	SNP	Forward (5′-3′)	Reverse (5′-3′)	Sequencing (5′-3′)	Ta
COMT	rs4680	tcaccatcgagatcaacccc	acaacgggtcaggcatgca	tggtggatttcgctg	62º
HCRTR1	rs2271933	atccagagtcacacaggcagaaa	tccttgcagagccgatgct	tgctcagagattttgga	58º
HCRTR2	rs2653349	tgtggcggctgaaataaag	tcatctggcctgacaaggtatcta	gcccggatgttgatg	58º

Table 2b. PCR primers and extension primers used in Sequenom

Gene	SNP	Oligo Sequence	Oligo Sequence2	Extension primer
COMT	rs4680	acgttggatgttttccaggtctgacaacgg	acgttggatgacccagcggatggtggattt	gtgtggatttcgctggc
HCRTR2	rs2653349	acgttggatgataaagcagatccgagcccag	acgttggatggatagcaaattgcaaatacc	agcacattgcaaataccaaaagcacaa

Statistical analysis

For statistical analysis logistic regression (Paper IV), chi-square analysis and Fisher's exact tests (Paper V), and linear regression (Paper VI) were used. Hardy-Weinberg equilibrium was checked in all control samples by comparing the observed genotype frequencies with the expected ones using chi-square analyses. Levels of significance were corrected for multiple comparisons using Bonferroni corrections.

References

Abelson JL, Nesse RM (1994): Pentagastrin infusions in patients with panic disorder. I. Symptoms and cardiovascular responses. *Biol Psychiatry* 36:73-83.

Abelson JL, Nesse RM, Weg JG, Curtis GC (1996): Respiratory psychophysiology and anxiety: cognitive intervention in the doxapram model of panic. *Psychosom Med* 58:302-313.

Abelson JL, Weg JG, Nesse RM, Curtis GC (2001): Persistent respiratory irregularity in patients with panic disorder. *Biol Psychiatry* 49:588-595.

Abrams K, Rassovsky Y, Kushner MG (2006): Evidence for respiratory and nonrespiratory subtypes in panic disorder. *Depress Anxiety* 23:474-481.

Adamantidis A, de Lecea L (2008): Physiological arousal: a role for hypothalamic systems. *Cell Mol Life Sci* 65:1475-1488.

Adamson PB, Vanoli E (2001): Early autonomic and repolarization abnormalities contribute to lethal arrhythmias in chronic ischemic heart failure: characteristics of a novel heart failure model in dogs with postmyocardial infarction left ventricular dysfunction. *J Am Coll Cardiol* 37:1741-1748.

Aghajanian GK, Graham AW, Sheard MH (1970): Serotonin-containing neurons in brain: depression of firing by monoamine oxidase inhibitors. *Science* 169:1100-1102.

Andersch S, Rosenberg NK, Kullingsjo H, Ottosson JO, Bech P, Bruun-Hansen J, et al (1991): Efficacy and safety of alprazolam, imipramine and placebo in treating panic disorder. A Scandinavian multicenter study. *Acta Psychiatr Scand Suppl* 365:18-27.

Andersson G, Bergstrom J, Carlbring P, Lindefors N (2005): The use of the Internet in the treatment of anxiety disorders. *Curr Opin Psychiatry* 18:73-77.

Annerbrink K, Olsson M, Melchior LK, Hedner J, Eriksson E (2003): Serotonin depletion increases respiratory variability in freely moving rats: implications for panic disorder. *Int J Neuropsychopharmacol* 6:51-56.

Arborelius L, Nomikos GG, Hertel P, Salmi P, Grillner P, Hook BB, et al (1996): The 5-HT1A receptor antagonist (S)-UH-301 augments the increase in extracellular concentrations of 5-HT in the frontal cortex produced by both acute and chronic treatment with citalopram. *Naunyn Schmiedebergs Arch Pharmacol* 353:630-640.

Atlas SA (2007): The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. *J Manag Care Pharm* 13:9-20.

Bach KB, Lutcavage ME, Mitchell GS (1993): Serotonin is necessary for short-term modulation of the exercise ventilatory response. *Respir Physiol* 91:57-70.

Ballenger JC, Wheadon DE, Steiner M, Bushnell W, Gergel IP (1998): Double-blind, fixed-dose, placebo-controlled study of paroxetine in the treatment of panic disorder. *Am J Psychiatry* 155:36-42.

Bandelow B, Saleh K, Pauls J, Domschke K, Wedekind D, Falkai P (2007): Insertion/deletion polymorphism in the gene for angiotensin converting enzyme (ACE) in panic disorder: A gender-specific effect? *World J Biol Psychiatry*:1-5.

Barlow DH, Gorman JM, Shear MK, Woods SW (2000): Cognitive-behavioral therapy, imipramine, or their combination for panic disorder: A randomized controlled trial. *JAMA* 283:2529-2536.

Bass C, Lelliott P, Marks I (1989): Fear talk versus voluntary hyperventilation in agoraphobics and normals: a controlled study. *Psychol Med* 19:669-676.

Beck JG, Shipherd JC, Ohtake P (2000): Do panic symptom profiles influence response to a hypoxic challenge in patients with panic disorder? A preliminary report. *Psychosom Med* 62:678-683.

Bell CC, Hildreth CJ, Jenkins EJ, Carter C (1988): The relationship of isolated sleep paralysis and panic disorder to hypertension. *J Natl Med Assoc* 80:289-294.

Bertani A, Perna G, Arancio C, Caldirola D, Bellodi L (1997): Pharmacologic effect of imipramine, paroxetine, and sertraline on 35% carbon dioxide hypersensitivity in panic patients: a double-blind, random, placebo-controlled study. *J Clin Psychopharmacol* 17:97-101.

Berthoud HR, Patterson LM, Sutton GM, Morrison C, Zheng H (2005): Orexin inputs to caudal raphe neurons involved in thermal, cardiovascular, and gastrointestinal regulation. *Histochem Cell Biol* 123:147-156.

Biber B, Alkin T (1999): Panic disorder subtypes: differential responses to CO2 challenge. *Am J Psychiatry* 156:739-744.

Bigos KL, Pollock BG, Aizenstein HJ, Fisher PM, Bies RR, Hariri AR (2008): Acute 5-HT Reuptake Blockade Potentiates Human Amygdala Reactivity. *Neuropsychopharmacology*.

Bilder RM, Volavka J, Czobor P, Malhotra AK, Kennedy JL, Ni X, et al (2002): Neurocognitive correlates of the COMT Val(158)Met polymorphism in chronic schizophrenia. *Biol Psychiatry* 52:701-707.

Bilder RM, Volavka J, Lachman HM, Grace AA (2004): The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* 29:1943-1961.

Blaya C, Salum GA, Lima MS, Leistner-Segal S, Manfro GG (2007): Lack of association between the Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Panic Disorder: a systematic review and meta-analysis. *Behav Brain Funct* 3:41.

Blier P, de Montigny C (1985): Serotoninergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. *Neuroscience* 16:949-955.

Blier P, de Montigny C (1990): Electrophysiological investigation of the adaptive response of the 5-HT system to the administration of 5-HT1A receptor agonists. *J Cardiovasc Pharmacol* 15 Suppl 7:S42-48.

Bocola V, Trecco MD, Fabbrini G, Paladini C, Sollecito A, Martucci N (1998): Antipanic effect of fluoxetine measured by CO2 challenge test. *Biol Psychiatry* 43:612-615.

Bystritsky A, Craske M, Maidenberg E, Vapnik T, Shapiro D (2000): Autonomic reactivity of panic patients during a CO2 inhalation procedure. *Depress Anxiety* 11:15-26.

Bystritsky A, Shapiro D (1992): Continuous physiological changes and subjective reports in panic patients: a preliminary methodological report. *Biol Psychiatry* 32:766-777.

Carr RE (1998): Panic disorder and asthma: causes, effects and research implications. *J Psychosom Res* 44:43-52.

Carr RE, Lehrer PM, Rausch LL, Hochron SM (1994): Anxiety sensitivity and panic attacks in an asthmatic population. *Behav Res Ther* 32:411-418.

Channick BJ, Adlin EV, Marks AD, Denenberg BS, McDonough MT, Chakko CS, et al (1981): Hyperthyroidism and mitral-valve prolapse. *N Engl J Med* 305:497-500.

Charney DS, Woods SW, Goodman WK, Heninger GR (1987): Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology (Berl)* 92:14-24.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, et al (2004): Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* 75:807-821.

Coates EL, Li A, Nattie EE (1993): Widespread sites of brain stem ventilatory chemoreceptors. *J Appl Physiol* 75:5-14.

Coplan JD, Gorman JM, Klein DF (1992): Serotonin related functions in panic-anxiety: a critical overview. *Neuropsychopharmacology* 6:189-200.

Creveling CR (2003): The role of catechol-O-methyltransferase in the inactivation of catecholestrogen. *Cell Mol Neurobiol* 23:289-291.

Crowe RR, Noyes R, Pauls DL, Slymen D (1983): A family study of panic disorder. *Arch Gen Psychiatry* 40:1065-1069.

Davidson JR (1998): The long-term treatment of panic disorder. *J Clin Psychiatry* 59 Suppl 8:17-21; discussion 22-13.

Davies SJ, Ghahramani P, Jackson PR, Noble TW, Hardy PG, Hippisley-Cox J, et al (1999): Association of panic disorder and panic attacks with hypertension. *Am J Med* 107:310-316.

de Beurs E, van Balkom AJ, Lange A, Koele P, van Dyck R (1995): Treatment of panic disorder with agoraphobia: comparison of fluvoxamine, placebo, and psychological panic management combined with exposure and of exposure in vivo alone. *Am J Psychiatry* 152:683-691.

de Bruyne MC, Kors JA, Hoes AW, Klootwijk P, Dekker JM, Hofman A, et al (1999): Both decreased and increased heart rate variability on the standard 10-second electrocardiogram predict cardiac mortality in the elderly: the Rotterdam Study. *Am J Epidemiol* 150:1282-1288.

de Frias CM, Annerbrink K, Westberg L, Eriksson E, Adolfsson R, Nilsson LG (2005): Catechol O-methyltransferase Val158Met polymorphism is associated with cognitive performance in nondemented adults. *J Cogn Neurosci* 17:1018-1025.

de Frias CM, arklund P, Eriksson E, Larsson A, Öman L, Annerbrink K, et al (2008): Influence of COMT gene polymorphism on fMRI-assessed sustained and transient
activity during a working memory task. *Manuscript*.

de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, et al (1998): The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* 95:322-327.

Deckert J, Nothen MM, Franke P, Delmo C, Fritze J, Knapp M, et al (1998): Systematic mutation screening and association study of the A1 and A2a adenosine receptor genes in panic disorder suggest a contribution of the A2a gene to the development of disease. *Mol Psychiatry* 3:81-85.

Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, et al (2000): Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Atherosclerosis Risk In Communities. *Circulation* 102:1239-1244.

Den Boer JA, Westenberg HG (1988): Effect of a serotonin and noradrenaline uptake inhibitor in panic disorder; a double-blind comparative study with fluvoxamine and maprotiline. *Int Clin Psychopharmacol* 3:59-74.

Diamond A, Briand L, Fossella J, Gehlbach L (2004): Genetic and neurochemical modulation of prefrontal cognitive functions in children. *Am J Psychiatry* 161:125-132.

Domschke K, Deckert J, O'Donovan M C, Glatt SJ (2007): Meta-analysis of COMT val158met in panic disorder: ethnic heterogeneity and gender specificity. *Am J Med Genet B Neuropsychiatr Genet* 144B:667-673.

Domschke K, Freitag CM, Kuhlenbaumer G, Schirmacher A, Sand P, Nyhuis P, et al (2004): Association of the functional V158M catechol-O-methyl-transferase polymorphism with panic disorder in women. *Int J Neuropsychopharmacol* 7:183-188.

Domschke K, Ohrmann P, Braun M, Suslow T, Bauer J, Hohoff C, et al (2008): Influence of the catechol-O-methyltransferase val158met genotype on amygdala and prefrontal cortex emotional processing in panic disorder. *Psychiatry Res* 163:13-20.

Drabant EM, Hariri AR, Meyer-Lindenberg A, Munoz KE, Mattay VS, Kolachana BS, et al (2006): Catechol O-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. *Arch Gen Psychiatry* 63:1396-1406.

Eaton WW, Kessler RC, Wittchen HU, Magee WJ (1994): Panic and panic disorder in the United States. *Am J Psychiatry* 151:413-420.

Eden S, Bolle P, Modigh K (1979): Monoaminergic control of episodic growth hormone secretion in the rat: effects of reserpine, alpha-methyl-p-tyrosine, p-chlorophenylalanine, and haloperidol. *Endocrinology* 105:523-529.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al (2001): Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 98:6917-6922.

Elam M, Yao T, Svensson TH, Thoren P (1984): Regulation of locus coeruleus neurons and splanchnic, sympathetic nerves by cardiovascular afferents. *Brain Res* 290:281-287.

Elam M, Yao T, Thoren P, Svensson TH (1981): Hypercapnia and hypoxia: chemoreceptor-mediated control of locus coeruleus neurons and splanchnic, sympathetic nerves. *Brain Res* 222:373-381.

Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, et al (1998): Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *J Comp Neurol* 402:442-459.

Enoch MA, Xu K, Ferro E, Harris CR, Goldman D (2003): Genetic origins of anxiety in women: a role for a functional catechol-O-methyltransferase polymorphism. *Psychiatr Genet* 13:33-41.

Erhardt A, Lucae S, Kern N, Unschuld PG, Ising M, Lieb R, et al (2008): Association of polymorphisms in the angiotensin-converting enzyme gene with syndromal panic attacks. *Mol Psychiatry* 13:242-243.

Eriksson E, Engberg G, Bing O, Nissbrandt H (1999): Effects of mCPP on the extracellular concentrations of serotonin and dopamine in rat brain. *Neuropsychopharmacology* 20:287-296.

Esler MD (1993): Catecholamines and essential hypertension. *Baillieres Clin Endocrinol Metab* 7:415-438.

Faravelli C, Abrardi L, Bartolozzi D, Cecchi C, Cosci F, D'Adamo D, et al (2004): The Sesto Fiorentino study: point and one-year prevalences of psychiatric disorders in an Italian community sample using clinical interviewers. *Psychother Psychosom* 73:226-234.

Feldman JL, Mitchell GS, Nattie EE (2003): Breathing: rhythmicity, plasticity, chemosensitivity. *Annu Rev Neurosci* 26:239-266.

Filho AS, Maciel BC, Martin-Santos R, Romano MM, Crippa JA (2008): Does the association between mitral valve prolapse and panic disorder really exist? *Prim Care Companion J Clin Psychiatry* 10:38-47.

Fornal CA, Metzler CW, Gallegos RA, Veasey SC, McCreary AC, Jacobs BL (1996): WAY-100635, a potent and selective 5-hydroxytryptamine1A antagonist, increases serotonergic neuronal activity in behaving cats: comparison with (S)-WAY-100135. *J Pharmacol Exp Ther* 278:752-762.

Forster EA, Cliffe IA, Bill DJ, Dover GM, Jones D, Reilly Y, et al (1995): A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. *Eur J Pharmacol* 281:81-88.

Freire RC, Lopes FL, Valenca AM, Nascimento I, Veras AB, Mezzasalma MA, et al (2008): Panic disorder respiratory subtype: a comparison between responses to hyperventilation and CO2 challenge tests. *Psychiatry Res* 157:307-310.

Fukunaka Y, Shinkai T, Hwang R, Hori H, Utsunomiya K, Sakata S, et al (2007): The orexin 1 receptor (HCRTR1) gene as a susceptibility gene contributing to polydipsia-hyponatremia in schizophrenia. *Neuromolecular Med* 9:292-297.

Fung SJ, Yamuy J, Sampogna S, Morales FR, Chase MH (2001): Hypocretin (orexin) input to trigeminal and hypoglossal motoneurons in the cat: a double-labeling immunohistochemical study. *Brain Res* 903:257-262.

Garakani A, Martinez JM, Aaronson CJ, Voustianiouk A, Kaufmann H, Gorman JM (2008): Effect of medication and psychotherapy on heart rate variability in panic disorder. *Depress Anxiety*.

Garattini S, Mennini T, Samanin R (1987): From fenfluramine racemate to d-fenfluramine. Specificity and potency of the effects on the serotoninergic system and food intake. *Ann N Y Acad Sci* 499:156-166.

Garssen B, Buikhuisen M, van Dyck R (1996): Hyperventilation and panic attacks. *Am J Psychiatry* 153:513-518.

Geraci M, Anderson TS, Slate-Cothren S, Post RM, McCann UD (2002): Pentagastrin-induced sleep panic attacks: panic in the absence of elevated baseline arousal. *Biol Psychiatry* 52:1183-1189.

Gillette PC, Smith RT, Garson A, Jr., Mullins CE, Gutgesell HP, Goh TH, et al (1985): Chronic supraventricular tachycardia. A curable cause of congestive cardiomyopathy. *JAMA* 253:391-392.

Goddard AW, Sholomskas DE, Walton KE, Augeri FM, Charney DS, Heninger GR, et al (1994): Effects of tryptophan depletion in panic disorder. *Biol Psychiatry* 36:775-777.

Goldberg C (2001): Cognitive processes in panic disorder: an extension of current models. *Psychol Rep* 88:139-159.

Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, et al (2003): Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. *Arch Gen Psychiatry* 60:889-896.

Goldstein RB, Weissman MM, Adams PB, Horwath E, Lish JD, Charney D, et al (1994): Psychiatric disorders in relatives of probands with panic disorder and/or major depression. *Arch Gen Psychiatry* 51:383-394.

Gorman JM, Askanazi J, Liebowitz MR, Fyer AJ, Stein J, Kinney JM, et al (1984): Response to hyperventilation in a group of patients with panic disorder. *Am J Psychiatry* 141:857-861.

Gorman JM, Browne ST, Papp LA, Martinez J, Welkowitz L, Coplan JD, et al (1997): Effect of antipanic treatment on response to carbon dioxide. *Biol Psychiatry* 42:982-991.

Gorman JM, Fyer MR, Goetz R, Askanazi J, Liebowitz MR, Fyer AJ, et al (1988a): Ventilatory physiology of patients with panic disorder. *Arch Gen Psychiatry* 45:31-39.

Gorman JM, Goetz RR, Fyer M, King DL, Fyer AJ, Liebowitz MR, et al (1988b): The mitral valve prolapse--panic disorder connection. *Psychosom Med* 50:114-122.

Gorman JM, Kent J, Martinez J, Browne S, Coplan J, Papp LA (2001): Physiological changes during carbon dioxide inhalation in patients with panic disorder, major depression, and premenstrual dysphoric disorder: evidence for a central fear mechanism. *Arch Gen Psychiatry* 58:125-131.

Gorman JM, Kent JM, Sullivan GM, Coplan JD (2000): Neuroanatomical hypothesis of panic disorder, revised. *Am J Psychiatry* 157:493-505.

Grant BF, Hasin DS, Stinson FS, Dawson DA, Goldstein RB, Smith S, et al (2006): The epidemiology of DSM-IV panic disorder and agoraphobia in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *J Clin Psychiatry* 67:363-374.

Guyenet PG, Stornetta RL, Bayliss DA (2008): Retrotrapezoid nucleus and central chemoreception. *J Physiol* 586:2043-2048.

Hagen K, Pettersen E, Stovner LJ, Skorpen F, Holmen J, Zwart JA (2007): High systolic blood pressure is associated with Val/Val genotype in the catechol-o-methyltransferase gene. The Nord-Trondelag Health Study (HUNT). *Am J Hypertens* 20:21-26.

Halford JC, Cooper GD, Dovey TM (2004): The pharmacology of human appetite expression. *Curr Drug Targets* 5:221-240.

Hamilton SP, Slager SL, De Leon AB, Heiman GA, Klein DF, Hodge SE, et al (2004): Evidence for genetic linkage between a polymorphism in the adenosine 2A receptor and panic disorder. *Neuropsychopharmacology* 29:558-565.

Hamilton SP, Slager SL, Heiman GA, Deng Z, Haghighi F, Klein DF, et al (2002): Evidence for a susceptibility locus for panic disorder near the catechol-O-methyltransferase gene on chromosome 22. *Biol Psychiatry* 51:591-601.

Harmer CJ, Mackay CE, Reid CB, Cowen PJ, Goodwin GM (2006): Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biol Psychiatry* 59:816-820.

Hegel MT, Ferguson RJ (1997): Psychophysiological assessment of respiratory function in panic disorder: evidence for a hyperventilation subtype. *Psychosom Med* 59:224-230.

Hettema JM, An SS, Bukszar J, van den Oord EJ, Neale MC, Kendler KS, et al (2008): Catechol-O-methyltransferase contributes to genetic susceptibility shared among anxiety spectrum phenotypes. *Biol Psychiatry* 64:302-310.

Hettema JM, Neale MC, Kendler KS (2001): A review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am J Psychiatry* 158:1568-1578.

Hjorth S (1993): Serotonin 5-HT1A autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study. *J Neurochem* 60:776-779.

Hofmann SG, Smits JA (2008): Cognitive-behavioral therapy for adult anxiety disorders: a meta-analysis of randomized placebo-controlled trials. *J Clin Psychiatry* 69:621-632.

Hollander E, Liebowitz MR, DeCaria C, Klein DF (1990): Fenfluramine, cortisol, and anxiety. *Psychiatry Res* 31:211-213.

Holtman JR, Jr., Dick TE, Berger AJ (1987): Serotonin-mediated excitation of recurrent laryngeal and phrenic motoneurons evoked by stimulation of the raphe obscurus. *Brain Res* 417:12-20.

Hood SD, Hince DA, Robinson H, Cirillo M, Christmas D, Kaye JM (2006): Serotonin regulation of the human stress response. *Psychoneuroendocrinology* 31:1087-1097.

Invernizzi R, Bramante M, Samanin R (1996): Role of 5-HT1A receptors in the effects of acute chronic fluoxetine on extracellular serotonin in the frontal cortex. *Pharmacol Biochem Behav* 54:143-147.

Jacob RG, Furman JM, Durrant JD, Turner SM (1996): Panic, agoraphobia, and vestibular dysfunction. *Am J Psychiatry* 153:503-512.

Jacobi F, Wittchen HU, Holting C, Hofler M, Pfister H, Muller N, et al (2004): Prevalence, co-morbidity and correlates of mental disorders in the general population: results from the German Health Interview and Examination Survey (GHS). *Psychol Med* 34:597-611.

Jennings DB (1994): The physicochemistry of [H+] and respiratory control: roles of PCO2, strong ions, and their hormonal regulators. *Can J Physiol Pharmacol* 72:1499-1512.

Joober R, Gauthier J, Lal S, Bloom D, Lalonde P, Rouleau G, et al (2002): Catechol-O-methyltransferase Val-108/158-Met gene variants associated with performance on the Wisconsin Card Sorting Test. *Arch Gen Psychiatry* 59:662-663.

Jungling K, Seidenbecher T, Sosulina L, Lesting J, Sangha S, Clark SD, et al (2008): Neuropeptide S-mediated control of fear expression and extinction: role of intercalated GABAergic neurons in the amygdala. *Neuron* 59:298-310.

Kahn JP, Drusin RE, Klein DF (1987a): Idiopathic cardiomyopathy and panic disorder: clinical association in cardiac transplant candidates. *Am J Psychiatry* 144:1327-1330.

Kahn RS, Westenberg HG, Verhoeven WM, Gispen-de Wied CC, Kamerbeek WD (1987b): Effect of a serotonin precursor and uptake inhibitor in anxiety disorders; a double-blind comparison of 5-hydroxytryptophan, clomipramine and placebo. *Int Clin Psychopharmacol* 2:33-45.

Kamide K, Kokubo Y, Yang J, Matayoshi T, Inamoto N, Takiuchi S, et al (2007): Association of genetic polymorphisms of ACADSB and COMT with human hypertension. *J Hypertens* 25:103-110.

Kandel ER (1983): From metapsychology to molecular biology: explorations into the nature of anxiety. *Am J Psychiatry* 140:1277-1293.

Kaplan DS, Masand PS, Gupta S (1996): The relationship of irritable bowel syndrome (IBS) and panic disorder. *Ann Clin Psychiatry* 8:81-88.

Kasamo K, Suzuki T, Tada K, Ueda N, Matsuda E, Ishikawa K, et al (2001): Endogenous 5-HT tonically inhibits spontaneous firing activity of dorsal hippocampus CA1 pyramidal neurons through stimulation of 5-HT(1A) receptors in quiet awake rats: in vivo electrophysiological evidence. *Neuropsychopharmacology* 24:141-151.

Katon W (1984): Panic disorder and somatization. Review of 55 cases. *Am J Med* 77:101-106.

Katon W, Roy-Byrne PP (1989): Panic disorder in the medically ill. *J Clin Psychiatry* 50:299-302.

Katschnig H, Amering M (1998): The long-term course of panic disorder and its predictors. *J Clin Psychopharmacol* 18:6S-11S.

Kawachi I, Sparrow D, Vokonas PS, Weiss ST (1995): Decreased heart rate variability in men with phobic anxiety (data from the Normative Aging Study). *Am J Cardiol* 75:882-885.

Kayaba Y, Nakamura A, Kasuya Y, Ohuchi T, Yanagisawa M, Komuro I, et al (2003): Attenuated defense response and low basal blood pressure in orexin knockout mice. *Am J Physiol Regul Integr Comp Physiol* 285:R581-593.

Kent JM, Coplan JD, Martinez J, Karmally W, Papp LA, Gorman JM (1996): Ventilatory effects of tryptophan depletion in panic disorder: a preliminary report. *Psychiatry Res* 64:83-90.

Kent JM, Papp LA, Martinez JM, Browne ST, Coplan JD, Klein DF, et al (2001): Specificity of panic response to CO(2) inhalation in panic disorder: a comparison with major depression and premenstrual dysphoric disorder. *Am J Psychiatry* 158:58-67.

Kessler RC, Greenberg PE, Mickelson KD, Meneades LM, Wang PS (2001): The effects of chronic medical conditions on work loss and work cutback. *J Occup Environ Med* 43:218-225.

Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al (1994): Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry* 51:8-19.

Kiropoulos LA, Klein B, Austin DW, Gilson K, Pier C, Mitchell J, et al (2008): Is internet-based CBT for panic disorder and agoraphobia as effective as face-to-face CBT? *J Anxiety Disord*.

Klein DF (1993): False suffocation alarms, spontaneous panics, and related conditions. An integrative hypothesis. *Arch Gen Psychiatry* 50:306-317.

Klein E, Cnaani E, Harel T, Braun S, Ben-Haim SA (1995): Altered heart rate variability in panic disorder patients. *Biol Psychiatry* 37:18-24.

Knott V, Bakish D, Lusk S, Barkely J (1997): Relaxation-induced EEG alterations in panic disorder patients. *J Anxiety Disord* 11:365-376.

Kuwaki T (2008): Orexinergic modulation of breathing across vigilance states. *Respir Physiol Neurobiol*.

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996): Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 6:243-250.

Laferrere B, Wurtman RJ (1989): Effect of D-fenfluramine on serotonin release in brains of anaesthetized rats. *Brain Res* 504:258-263.

Lalley PM (1986): Serotoninergic and non-serotoninergic responses of phrenic motoneurones to raphe stimulation in the cat. *J Physiol* 380:373-385.

Lee YJ, Curtis GC, Weg JG, Abelson JL, Modell JG, Campbell KM (1993): Panic attacks induced by doxapram. *Biol Psychiatry* 33:295-297.

Lewandowski KE (2007): Relationship of catechol-O-methyltransferase to schizophrenia and its correlates: evidence for associations and complex interactions. *Harv Rev Psychiatry* 15:233-244.

Ley R (1985): Agoraphobia, the panic attack and the hyperventilation syndrome. *Behav Res Ther* 23:79-81.

Liebowitz MR, Fyer AJ, Gorman JM, Dillon D, Appleby IL, Levy G, et al (1984): Lactate provocation of panic attacks. I. Clinical and behavioral findings. *Arch Gen Psychiatry* 41:764-770.

Loeschcke HH (1973): Respiratory chemosensitivity in the medulla oblongata. *Acta Neurobiol Exp (Wars)* 33:97-112.

Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, et al (1995): Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 34:4202-4210.

Louet JF, LeMay C, Mauvais-Jarvis F (2004): Antidiabetic actions of estrogen: insight from human and genetic mouse models. *Curr Atheroscler Rep* 6:180-185.

Maddock RJ, Buonocore MH, Copeland LE, Richards AL (2008): Elevated brain lactate responses to neural activation in panic disorder: a dynamic 1H-MRS study. *Mol Psychiatry*.

Maddock RJ, Carter CS (1991): Hyperventilation-induced panic attacks in panic disorder with agoraphobia. *Biol Psychiatry* 29:843-854.

Maier W, Lichtermann D, Minges J, Oehrlein A, Franke P (1993): A controlled family study in panic disorder. *J Psychiatr Res* 27 Suppl 1:79-87.

Malhotra AK, Kestler LJ, Mazzanti C, Bates JA, Goldberg T, Goldman D (2002): A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. *Am J Psychiatry* 159:652-654.

Manzke T, Guenther U, Ponimaskin EG, Haller M, Dutschmann M, Schwarzacher S, et al (2003): 5-HT4(a) receptors avert opioid-induced breathing depression without loss of analgesia. *Science* 301:226-229.

Marazziti D, Toni C, Pedri S, Bonuccelli U, Pavese N, Lucetti C, et al (1999): Prevalence of headache syndromes in panic disorder. *Int Clin Psychopharmacol* 14:247-251.

Markowitz JS, Weissman MM, Ouellette R, Lish JD, Klerman GL (1989): Quality of life in panic disorder. *Arch Gen Psychiatry* 46:984-992.

Maron E, Kuikka JT, Shlik J, Vasar V, Vanninen E, Tiihonen J (2004): Reduced brain serotonin transporter binding in patients with panic disorder. *Psychiatry Res* 132:173-181.

Martin-Body RL, Grundy HR (1985): Effects of neurotoxin-induced brainstem lesions on the respiratory responses of conscious rats. *Clin Exp Pharmacol Physiol* 12:427-437.
Martinez JM, Kent JM, Coplan JD, Browne ST, Papp LA, Sullivan GM, et al (2001): Respiratory variability in panic disorder. *Depress Anxiety* 14:232-237.
Massion AO, Warshaw MG, Keller MB (1993): Quality of life and psychiatric morbidity in panic disorder and generalized anxiety disorder. *Am J Psychiatry* 150:600-607.
Mattay VS, Goldberg TE, Fera F, Hariri AR, Tessitore A, Egan MF, et al (2003): Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc Natl Acad Sci U S A* 100:6186-6191.
McCann UD, Slate SO, Geraci M, Roscow-Terrill D, Uhde TW (1997): A comparison of the effects of intravenous pentagastrin on patients with social phobia, panic disorder and healthy controls. *Neuropsychopharmacology* 16:229-237.
McCrimmon DR, Mitchell, G.S., Dekin, M.S. (1995): Glutamate, GABA, and serotonin in ventilatory control. In: Dempsey JA editor. *Regulation of Breathing*, Second edition ed. New York: Dekker, pp 151-217.
Meerabux J, Iwayama Y, Sakurai T, Ohba H, Toyota T, Yamada K, et al (2005): Association of an orexin 1 receptor 408Val variant with polydipsia-hyponatremia in schizophrenic subjects. *Biol Psychiatry* 58:401-407.
Merikangas KR, Angst J, Eaton W, Canino G, Rubio-Stipec M, Wacker H, et al (1996): Comorbidity and boundaries of affective disorders with anxiety disorders and substance misuse: results of an international task force. *Br J Psychiatry Suppl*:58-67.
Meuret AE, White KS, Ritz T, Roth WT, Hofmann SG, Brown TA (2006): Panic attack symptom dimensions and their relationship to illness characteristics in panic disorder. *J Psychiatr Res* 40:520-527.
Meuret AE, Wilhelm FH, Roth WT (2004): Respiratory feedback for treating panic disorder. *J Clin Psychol* 60:197-207.
Michelson D, Lydiard RB, Pollack MH, Tamura RN, Hoog SL, Tepner R, et al (1998): Outcome assessment and clinical improvement in panic disorder: evidence from a randomized controlled trial of fluoxetine and placebo. The Fluoxetine Panic Disorder Study Group. *Am J Psychiatry* 155:1570-1577.
Miller HE, Deakin JF, Anderson IM (2000): Effect of acute tryptophan depletion on CO2-induced anxiety in patients with panic disorder and normal volunteers. *Br J Psychiatry* 176:182-188.
Millhorn DE, Eldridge FL, Waldrop TG (1980): Prolonged stimulation of respiration by endogenous central serotonin. *Respir Physiol* 42:171-188.
Millhorn DE, Eldridge FL, Waldrop TG, Klingler LE (1983): Centrally and peripherally administered 5-HTP have opposite effects on respiration. *Brain Res* 264:349-354.
Mitchell GS, Smith CA, Vidruk EH, Jameson LC, Dempsey JA (1983): Effects of p-chlorophenylalanine on ventilatory control in goats. *J Appl Physiol* 54:277-283.
Mitchell RA (1969): Respiratory chemosensitivity in the medulla oblongata. *J Physiol* 202:3P-4P.
Mitte K (2005): A meta-analysis of the efficacy of psycho- and pharmacotherapy in panic disorder with and without agoraphobia. *J Affect Disord* 88:27-45.
Mlinar B, Tatini F, Ballini C, Nencioni S, Della Corte L, Corradetti R (2005): Differential autoinhibition of 5-hydroxytryptamine neurons by 5-hydroxytryptamine in the dorsal raphe nucleus. *Neuroreport* 16:1351-1355.
Modigh K (1987): Antidepressant drugs in anxiety disorders. *Acta Psychiatr Scand Suppl* 335:57-74.

Modigh K, Westberg P, Eriksson E (1992): Superiority of clomipramine over imipramine in the treatment of panic disorder: a placebo-controlled trial. *J Clin Psychopharmacol* 12:251-261.

Mortimore C, Anderson IM (2000): d-Fenfluramine in panic disorder: a dual role for 5-hydroxytryptamine. *Psychopharmacology (Berl)* 149:251-258.

Moynihan JE, Gevirtz RN (2001): Respiratory and cognitive subtypes of panic. Preliminary validation of Ley's model. *Behav Modif* 25:555-583.

Mueller RA, Lundberg D, Breese G (1980): Effects of different monoamine oxidase inhibitors on respiratory activity in rats with chronically impaired central serotonergic function. *Acta Pharmacol Toxicol (Copenh)* 47:285-293.

Mulkey DK, Stornetta RL, Weston MC, Simmons JR, Parker A, Bayliss DA, et al (2004): Respiratory control by ventral surface chemoreceptor neurons in rats. *Nat Neurosci* 7:1360-1369.

Muller M, van der Schouw YT, Thijssen JH, Grobbee DE (2003): Endogenous sex hormones and cardiovascular disease in men. *J Clin Endocrinol Metab* 88:5076-5086.

Murakoshi T, Suzue T, Tamai S (1985): A pharmacological study on respiratory rhythm in the isolated brainstem-spinal cord preparation of the newborn rat. *Br J Pharmacol* 86:95-104.

Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, Goto K (1999): Distribution of orexin neurons in the adult rat brain. *Brain Res* 827:243-260.

Nardi AE, Freire RC, Zin WA (2008): Panic disorder and control of breathing. *Respir Physiol Neurobiol*.

Nardi AE, Valenca AM, Lopes FL, de-Melo-Neto VL, Freire RC, Veras AB, et al (2007): Caffeine and 35% carbon dioxide challenge tests in panic disorder. *Hum Psychopharmacol* 22:231-240.

Nardi AE, Valenca AM, Lopes FL, Nascimento I, Veras AB, Freire RC, et al (2006): Psychopathological profile of 35% CO2 challenge test-induced panic attacks: a comparison with spontaneous panic attacks. *Compr Psychiatry* 47:209-214.

Nash JR, Sargent PA, Rabiner EA, Hood SD, Argyropoulos SV, Potokar JP, et al (2008): Serotonin 5-HT1A receptor binding in people with panic disorder: positron emission tomography study. *Br J Psychiatry* 193:229-234.

Neumeister A, Bain E, Nugent AC, Carson RE, Bonne O, Luckenbaugh DA, et al (2004): Reduced serotonin type 1A receptor binding in panic disorder. *J Neurosci* 24:589-591.

Nordfors L, Jansson M, Sandberg G, Lavebratt C, Sengul S, Schalling M, et al (2002): Large-scale genotyping of single nucleotide polymorphisms by Pyrosequencingtrade mark and validation against the 5'nuclease (Taqman((R))) assay. *Hum Mutat* 19:395-401.

Nutt DJ, Glue P (1989): Clinical pharmacology of anxiolytics and antidepressants: a psychopharmacological perspective. *Pharmacol Ther* 44:309-334.

Okamura N, Hashimoto K, Iyo M, Shimizu E, Dempfle A, Friedel S, et al (2007): Gender-specific association of a functional coding polymorphism in the Neuropeptide S receptor gene with panic disorder but not with schizophrenia or attention-deficit/hyperactivity disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 31:1444-1448.

Olson EB, Jr. (1987): Ventilatory adaptation to hypoxia occurs in serotonin-depleted rats. *Respir Physiol* 69:227-235.

Olsson CA, Anney RJ, Lotfi-Miri M, Byrnes GB, Williamson R, Patton GC (2005): Association between the COMT Val158Met polymorphism and propensity to anxiety in an Australian population-based longitudinal study of adolescent health. *Psychiatr Genet* 15:109-115.

Olsson M, Annerbrink K, Bengtsson F, Hedner J, Eriksson E (2004a): Paroxetine influences respiration in rats: implications for the treatment of panic disorder. *Eur Neuropsychopharmacol* 14:29-37.

Olsson M, Annerbrink K, Hedner J, Eriksson E (2004b): Intracerebroventricular administration of the angiotensin II receptor antagonist saralasin reduces respiratory rate and tidal volume variability in freely moving Wistar rats. *Psychoneuroendocrinology* 29:107-112.

Olsson M, Annerbrink K, Westberg L, Melke J, Baghaei F, Rosmond R, et al (2004c): Angiotensin-related genes in patients with panic disorder. *Am J Med Genet B Neuropsychiatr Genet* 127B:81-84.

Onur E, Alkin T, Tural U (2007): Panic disorder subtypes: further clinical differences. *Depress Anxiety* 24:479-486.

Papp LA, Klein DF, Gorman JM (1993a): Carbon dioxide hypersensitivity, hyperventilation, and panic disorder. *Am J Psychiatry* 150:1149-1157.

Papp LA, Klein DF, Martinez J, Schneier F, Cole R, Liebowitz MR, et al (1993b): Diagnostic and substance specificity of carbon-dioxide-induced panic. *Am J Psychiatry* 150:250-257.

Papp LA, Martinez JM, Klein DF, Coplan JD, Norman RG, Cole R, et al (1997): Respiratory psychophysiology of panic disorder: three respiratory challenges in 98 subjects. *Am J Psychiatry* 154:1557-1565.

Perna G, Battaglia M, Garberi A, Arancio C, Bertani A, Bellodi L (1994): Carbon dioxide/oxygen challenge test in panic disorder. *Psychiatry Res* 52:159-171.

Perna G, Bertani A, Caldirola D, Smeraldi E, Bellodi L (2001): A comparison of citalopram and paroxetine in the treatment of panic disorder: a randomized, single-blind study. *Pharmacopsychiatry* 34:85-90.

Perna G, Bertani A, Gabriele A, Politi E, Bellodi L (1997): Modification of 35% carbon dioxide hypersensitivity across one week of treatment with clomipramine and fluvoxamine: a double-blind, randomized, placebo-controlled study. *J Clin Psychopharmacol* 17:173-178.

Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, et al (1998): Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 18:9996-10015.

Pillay SS, Gruber SA, Rogowska J, Simpson N, Yurgelun-Todd DA (2006): fMRI of fearful facial affect recognition in panic disorder: the cingulate gyrus-amygdala connection. *J Affect Disord* 94:173-181.

Pine DS, Weese-Mayer DE, Silvestri JM, Davies M, Whitaker AH, Klein DF (1994): Anxiety and congenital central hypoventilation syndrome. *Am J Psychiatry* 151:864-870.

Pitts FN, Jr., McClure JN, Jr. (1967): Lactate metabolism in anxiety neurosis. *N Engl J Med* 277:1329-1336.

Pohl R, Yeragani VK, Balon R, Lycaki H (1988): The jitteriness syndrome in panic disorder patients treated with antidepressants. *J Clin Psychiatry* 49:100-104.

Pohl RB, Wolkow RM, Clary CM (1998): Sertraline in the treatment of panic disorder: a double-blind multicenter trial. *Am J Psychiatry* 155:1189-1195.

Pollack MH, Kradin R, Otto MW, Worthington J, Gould R, Sabatino SA, et al (1996): Prevalence of panic in patients referred for pulmonary function testing at a major medical center. *Am J Psychiatry* 153:110-113.

Pols HJ, Hauzer RC, Meijer JA, Verburg K, Griez EJ (1996): Fluvoxamine attenuates panic induced by 35% CO2 challenge. *J Clin Psychiatry* 57:539-542.

Porzelius J, Vest M, Nochomovitz M (1992): Respiratory function, cognitions, and panic in chronic obstructive pulmonary patients. *Behav Res Ther* 30:75-77.

Rainero I, Rubino E, Valfre W, Gallone S, De Martino P, Zampella E, et al (2007): Association between the G1246A polymorphism of the hypocretin receptor 2 gene and cluster headache: a meta-analysis. *J Headache Pain* 8:152-156.

Rainey JM, Jr., Frohman CE, Warner K, Bates S, Pohl RB, Yeragani V (1985): Panic anxiety and lactate metabolism. *Psychopharmacol Bull* 21:434-437.

Rapee R (1986): Differential response to hyperventilation in panic disorder and generalized anxiety disorder. *J Abnorm Psychol* 95:24-28.

Rapee RM, Brown TA, Antony MM, Barlow DH (1992): Response to hyperventilation and inhalation of 5.5% carbon dioxide-enriched air across the DSM-III-R anxiety disorders. *J Abnorm Psychol* 101:538-552.

Richerson GB (2004): Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. *Nat Rev Neurosci* 5:449-461.

Richerson GB, Wang W, Hodges MR, Dohle CI, Diez-Sampedro A (2005): Homing in on the specific phenotype(s) of central respiratory chemoreceptors. *Exp Physiol* 90:259-266; discussion 266-259.

Richter DW, Manzke T, Wilken B, Ponimaskin E (2003): Serotonin receptors: guardians of stable breathing. *Trends Mol Med* 9:542-548.

Rizzi A, Vergura R, Marzola G, Ruzza C, Guerrini R, Salvadori S, et al (2008): Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. *Br J Pharmacol* 154:471-479.

Rosmond R, Bjorntorp P (1998): Psychiatric ill-health of women and its relationship to obesity and body fat distribution. *Obes Res* 6:338-345.

Rosmond R, Dallman MF, Bjorntorp P (1998): Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *J Clin Endocrinol Metab* 83:1853-1859.

Roth WT (2005): Physiological markers for anxiety: panic disorder and phobias. *Int J Psychophysiol* 58:190-198.

Rothe C, Koszycki D, Bradwejn J, King N, Deluca V, Tharmalingam S, et al (2006): Association of the Val158Met catechol O-methyltransferase genetic polymorphism with panic disorder. *Neuropsychopharmacology* 31:2237-2242.

Roy-Byrne PP, Stang P, Wittchen HU, Ustun B, Walters EE, Kessler RC (2000): Lifetime panic-depression comorbidity in the National Comorbidity Survey. Association with symptoms, impairment, course and help-seeking. *Br J Psychiatry* 176:229-235.

Sakurai T (2007): The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat Rev Neurosci* 8:171-181.

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al (1998): Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92:1 page following 696.

Sakurai T, Nagata R, Yamanaka A, Kawamura H, Tsujino N, Muraki Y, et al (2005): Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* 46:297-308.

Sapru HN, Krieger AJ (1977): Effect of 5-hydroxytryptamine on the peripheral chemoreceptors in the rat. *Res Commun Chem Pathol Pharmacol* 16:245-250.

Schruers K, Klaassen T, Pols H, Overbeek T, Deutz NE, Griez E (2000): Effects of tryptophan depletion on carbon dioxide provoked panic in panic disorder patients. *Psychiatry Res* 93:179-187.

Schurks M, Kurth T, Geissler I, Tessmann G, Diener HC, Rosskopf D (2006): Cluster headache is associated with the G1246A polymorphism in the hypocretin receptor 2 gene. *Neurology* 66:1917-1919.

Schwartz GE, Goetz RR, Klein DF, Endicott J, Gorman JM (1996): Tidal volume of respiration and "sighing" as indicators of breathing irregularities in panic disorder patients. *Anxiety* 2:145-148.

Severson CA, Wang W, Pieribone VA, Dohle CI, Richerson GB (2003): Midbrain serotonergic neurons are central pH chemoreceptors. *Nat Neurosci* 6:1139-1140.

Sharp T, Bramwell SR, Grahame-Smith DG (1989): 5-HT1 agonists reduce 5-hydroxytryptamine release in rat hippocampus in vivo as determined by brain microdialysis. *Br J Pharmacol* 96:283-290.

Shavitt RG, Gentil V, Mandetta R (1992): The association of panic/agoraphobia and asthma. Contributing factors and clinical implications. *Gen Hosp Psychiatry* 14:420-423.

Sheehan DV, Raj AB, Harnett-Sheehan K, Soto S, Knapp E (1993): The relative efficacy of high-dose buspirone and alprazolam in the treatment of panic disorder: a double-blind placebo-controlled study. *Acta Psychiatr Scand* 88:1-11.

Shekhar A, Johnson PL, Sajdyk TJ, Fitz SD, Keim SR, Kelley PE, et al (2006): Angiotensin-II is a putative neurotransmitter in lactate-induced panic-like responses in rats with disruption of GABAergic inhibition in the dorsomedial hypothalamus. *J Neurosci* 26:9205-9215.

Shlik J, Aluoja A, Vasar V, Vasar E, Podar T, Bradwejn J (1997): Effects of citalopram treatment on behavioural, cardiovascular and neuroendocrine response to cholecystokinin tetrapeptide challenge in patients with panic disorder. *J Psychiatry Neurosci* 22:332-340.

Sinha S, Papp LA, Gorman JM (2000): How study of respiratory physiology aided our understanding of abnormal brain function in panic disorder. *J Affect Disord* 61:191-200.

Smolka MN, Schumann G, Wrase J, Grusser SM, Flor H, Mann K, et al (2005): Catechol-O-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *J Neurosci* 25:836-842.

Smoller JW, Tsuang MT (1998): Panic and phobic anxiety: defining phenotypes for genetic studies. *Am J Psychiatry* 155:1152-1162.

Spinhoven P, Ros M, Westgeest A, Van der Does AJ (1994): The prevalence of respiratory disorders in panic disorder, major depressive disorder and V-code patients. *Behav Res Ther* 32:647-649.

Stahl SM (1998): Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. *J Affect Disord* 51:215-235.

Stahl SM, Gergel I, Li D (2003): Escitalopram in the treatment of panic disorder: a randomized, double-blind, placebo-controlled trial. *J Clin Psychiatry* 64:1322-1327.

Stein MB, Fallin MD, Schork NJ, Gelernter J (2005): COMT polymorphisms and anxiety-related personality traits. *Neuropsychopharmacology* 30:2092-2102.

Stein MB, Millar TW, Larsen DK, Kryger MH (1995): Irregular breathing during sleep in patients with panic disorder. *Am J Psychiatry* 152:1168-1173.

Stone RA, Barnes PJ, Chung KF (1997): Effect of 5-HT1A receptor agonist, 8-OH-DPAT, on cough responses in the conscious guinea pig. *Eur J Pharmacol* 332:201-207.

Struzik L, Duffin J, Vermani M, Hegadoren K, Katzman MA (2002): Effects of tryptophan depletion on central and peripheral chemoreflexes in man. *Respir Physiol Neurobiol* 133:183-195.

Sullivan GM, Kent JM, Kleber M, Martinez JM, Yeragani VK, Gorman JM (2004): Effects of hyperventilation on heart rate and QT variability in panic disorder pre- and post-treatment. *Psychiatry Res* 125:29-39.

Svensson TH (1987): Peripheral, autonomic regulation of locus coeruleus noradrenergic neurons in brain: putative implications for psychiatry and psychopharmacology. *Psychopharmacology (Berl)* 92:1-7.

Targum SD, Marshall LE (1989): Fenfluramine provocation of anxiety in patients with panic disorder. *Psychiatry Res* 28:295-306.

Taylor NC, Li A, Green A, Kinney HC, Nattie EE (2004): Chronic fluoxetine microdialysis into the medullary raphe nuclei of the rat, but not systemic administration, increases the ventilatory response to CO2. *J Appl Physiol* 97:1763-1773.

Tchernof A, Despres JP (2000): Sex steroid hormones, sex hormone-binding globulin, and obesity in men and women. *Horm Metab Res* 32:526-536.

Tesar GE (1990): High-potency benzodiazepines for short-term management of panic disorder: the U.S. experience. *J Clin Psychiatry* 51 Suppl:4-10; discussion 50-13.

Todd GP, Chadwick IG, Yeo WW, Jackson PR, Ramsay LE (1995): Pressor effect of hyperventilation in healthy subjects. *J Hum Hypertens* 9:119-122.

Truitt WA, Sajdyk TJ, Dietrich AD, Oberlin B, McDougle CJ, Shekhar A (2007): From anxiety to autism: spectrum of abnormal social behaviors modeled by progressive disruption of inhibitory neuronal function in the basolateral amygdala in Wistar rats. *Psychopharmacology (Berl)* 191:107-118.

Tsuji H, Larson MG, Venditti FJ, Jr., Manders ES, Evans JC, Feldman CL, et al (1996): Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* 94:2850-2855.

Tucker P, Adamson P, Miranda R, Jr., Scarborough A, Williams D, Groff J, et al (1997): Paroxetine increases heart rate variability in panic disorder. *J Clin Psychopharmacol* 17:370-376.

Tunbridge EM, Harrison PJ, Weinberger DR (2006): Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol Psychiatry* 60:141-151.

Tyrer P, Shawcross C (1988): Monoamine oxidase inhibitors in anxiety disorders. *J Psychiatr Res* 22 Suppl 1:87-98.

Uchida RR, Del-Ben CM, Busatto GF, Duran FL, Guimaraes FS, Crippa JA, et al (2008): Regional gray matter abnormalities in panic disorder: a voxel-based morphometry study. *Psychiatry Res* 163:21-29.

Wade AG, Lepola U, Koponen HJ, Pedersen V, Pedersen T (1997): The effect of citalopram in panic disorder. *Br J Psychiatry* 170:549-553.

van Apeldoorn FJ, van Hout WJ, Mersch PP, Huisman M, Slaap BR, Hale WW, 3rd, et al (2008): Is a combined therapy more effective than either CBT or SSRI alone? Results of a multicenter trial on panic disorder with or without agoraphobia. *Acta Psychiatr Scand* 117:260-270.

van den Boom D, Ehrich M (2007): Discovery and identification of sequence polymorphisms and mutations with MALDI-TOF MS. *Methods Mol Biol* 366:287-306.

van den Hout MA, Hoekstra R, Arntz A, Christiaanse M, Ranschaert W, Schouten E (1992): Hyperventilation is not diagnostically specific to panic patients. *Psychosom Med* 54:182-191.

van Megen HJ, Westenberg HG, den Boer JA, Haigh JR, Traub M (1994): Pentagastrin induced panic attacks: enhanced sensitivity in panic disorder patients. *Psychopharmacology (Berl)* 114:449-455.

van Megen HJ, Westenberg HG, den Boer JA, Slaap B, Scheepmakers A (1997): Effect of the selective serotonin reuptake inhibitor fluvoxamine on CCK-4 induced panic attacks. *Psychopharmacology (Berl)* 129:357-364.

van Vliet IM, Westenberg HG, den Boer JA (1996): Effects of the 5-HT1A receptor agonist flesinoxan in panic disorder. *Psychopharmacology (Berl)* 127:174-180.

Wang W, Pizzonia JH, Richerson GB (1998): Chemosensitivity of rat medullary raphe neurones in primary tissue culture. *J Physiol* 511 (Pt 2):433-450.
Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG, et al (1997): The cross-national epidemiology of panic disorder. *Arch Gen Psychiatry* 54:305-309.
Weissman MM, Markowitz JS, Ouellette R, Greenwald S, Kahn JP (1990): Panic disorder and cardiovascular/cerebrovascular problems: results from a community survey. *Am J Psychiatry* 147:1504-1508.
Weissman NJ, Shear MK, Kramer-Fox R, Devereux RB (1987): Contrasting patterns of autonomic dysfunction in patients with mitral valve prolapse and panic attacks. *Am J Med* 82:880-888.
Wilhelm FH, Trabert W, Roth WT (2001a): Characteristics of sighing in panic disorder. *Biol Psychiatry* 49:606-614.
Wilhelm FH, Trabert W, Roth WT (2001b): Physiologic instability in panic disorder and generalized anxiety disorder. *Biol Psychiatry* 49:596-605.
Wilkerson A, Levin ED (1999): Ventral hippocampal dopamine D1 and D2 systems and spatial working memory in rats. *Neuroscience* 89:743-749.
Wilkinson DJ, Thompson JM, Lambert GW, Jennings GL, Schwarz RG, Jefferys D, et al (1998): Sympathetic activity in patients with panic disorder at rest, under laboratory mental stress, and during panic attacks. *Arch Gen Psychiatry* 55:511-520.
Williams RH, Burdakov D (2008): Hypothalamic orexins/hypocretins as regulators of breathing. *Expert Rev Mol Med* 10:e28.
Winsky-Sommerer R, Yamanaka A, Diano S, Borok E, Roberts AJ, Sakurai T, et al (2004): Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. *J Neurosci* 24:11439-11448.
Vitale G, Filaferro M, Ruggieri V, Pennella S, Frigeri C, Rizzi A, et al (2008): Anxiolytic-like effect of neuropeptide S in the rat defensive burying. *Peptides*.
Wittchen HU, Nelson CB, Lachner G (1998a): Prevalence of mental disorders and psychosocial impairments in adolescents and young adults. *Psychol Med* 28:109-126.
Wittchen HU, Reed V, Kessler RC (1998b): The relationship of agoraphobia and panic in a community sample of adolescents and young adults. *Arch Gen Psychiatry* 55:1017-1024.
Vollrath M, Koch R, Angst J (1990): The Zurich Study. IX. Panic disorder and sporadic panic: symptoms, diagnosis, prevalence, and overlap with depression. *Eur Arch Psychiatry Neurol Sci* 239:221-230.
Woo JM, Yoon KS, Choi YH, Oh KS, Lee YS, Yu BH (2004): The association between panic disorder and the L/L genotype of catechol-O-methyltransferase. *J Psychiatr Res* 38:365-370.
Woo JM, Yoon KS, Yu BH (2002): Catechol O-methyltransferase genetic polymorphism in panic disorder. *Am J Psychiatry* 159:1785-1787.
Woods SW, Charney DS, Loke J, Goodman WK, Redmond DE, Jr., Heninger GR (1986): Carbon dioxide sensitivity in panic anxiety. Ventilatory and anxiogenic response to carbon dioxide in healthy subjects and patients with panic anxiety before and after alprazolam treatment. *Arch Gen Psychiatry* 43:900-909.
Yellowlees PM, Alpers JH, Bowden JJ, Bryant GD, Ruffin RE (1987): Psychiatric morbidity in patients with chronic airflow obstruction. *Med J Aust* 146:305-307.
Yellowlees PM, Haynes S, Potts N, Ruffin RE (1988): Psychiatric morbidity in patients with life-threatening asthma: initial report of a controlled study. *Med J Aust* 149:246-249.
Yeragani VK, Pohl R, Balon R, Jampala VC, Jayaraman A (2002a): Twenty-four-hour QT interval variability: increased QT variability during sleep in patients with panic disorder. *Neuropsychobiology* 46:1-6.

Yeragani VK, Pohl R, Berger R, Balon R, Ramesh C, Glitz D, et al (1993): Decreased heart rate variability in panic disorder patients: a study of power-spectral analysis of heart rate. *Psychiatry Res* 46:89-103.

Yeragani VK, Pohl R, Jampala VC, Balon R, Ramesh C, Srinivasan K (2000): Increased QT variability in patients with panic disorder and depression. *Psychiatry Res* 93:225-235.

Yeragani VK, Pohl R, Srinivasan K, Balon R, Ramesh C, Berchou R (1995): Effects of isoproterenol infusions on heart rate variability in patients with panic disorder. *Psychiatry Res* 56:289-293.

Yeragani VK, Radhakrishna RK, Tancer M, Uhde T (2002b): Nonlinear measures of respiration: respiratory irregularity and increased chaos of respiration in patients with panic disorder. *Neuropsychobiology* 46:111-120.

Yeragani VK, Rao R, Tancer M, Uhde T (2004): Paroxetine decreases respiratory irregularity of linear and nonlinear measures of respiration in patients with panic disorder. A preliminary report. *Neuropsychobiology* 49:53-57.

Yoshida K, McCormack S, Espana RA, Crocker A, Scammell TE (2006): Afferents to the orexin neurons of the rat brain. *J Comp Neurol* 494:845-861.

Young JK, Wu M, Manaye KF, Kc P, Allard JS, Mack SO, et al (2005): Orexin stimulates breathing via medullary and spinal pathways. *J Appl Physiol* 98:1387-1395.

Zandbergen J, Bright M, Pols H, Fernandez I, de Loof C, Griez EJ (1991): Higher lifetime prevalence of respiratory diseases in panic disorder? *Am J Psychiatry* 148:1583-1585.

Zandbergen J, Lousberg HH, Pols H, de Loof C, Griez EJ (1990): Hypercarbia versus hypocarbia in panic disorder. *J Affect Disord* 18:75-81.

Zandbergen J, van Aalst V, de Loof C, Pols H, Griez E (1993): No chronic hyperventilation in panic disorder patients. *Psychiatry Res* 47:1-6.

Zaubler TS, Katon W (1998): Panic disorder in the general medical setting. *J Psychosom Res* 44:25-42.

Zhang W, Fukuda Y, Kuwaki T (2005): Respiratory and cardiovascular actions of orexin-A in mice. *Neurosci Lett* 385:131-136.

Zhang W, Sakurai T, Fukuda Y, Kuwaki T (2006a): Orexin neuron-mediated skeletal muscle vasodilation and shift of baroreflex during defense response in mice. *Am J Physiol Regul Integr Comp Physiol* 290:R1654-1663.

Zhang W, Shimoyama M, Fukuda Y, Kuwaki T (2006b): Multiple components of the defense response depend on orexin: evidence from orexin knockout mice and orexin neuron-ablated mice. *Auton Neurosci* 126-127:139-145.