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SEROTONIN FUNCTION IN PANIC DISORDER: FROM CLINICAL EXPERIMENTS TO BRAIN IMAGING AND GENETICS

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ABBREVIATIONS

APEX	Arrayed Primer Extension
CCK-4	Cholecystokinin-tetrapeptide
CNS	Central nervous system
DA	Dopamine
dFEN	*d*-Fenfluramine
DRN	Dorsal raphe nucleus
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
GABA	gamma-aminobutyric acid
GAD	Generalized anxiety disorder
HAMA	Hamilton Anxiety Scale
LC	Locus coeruleus
MAO	Monoamine oxidase enzyme
m-CPP	m-Chlorophenylpiperazine
MINI	Mini International Neuropsychiatric Interview
MRI	Magnetic resonance imaging
MRN	Median raphe nucleus
NOR	Noradrenaline (norepinephrine)
nor-ß-CIT	Iodine-123 labeled 2 ß-carbomethoxy-3β-(4-iodophenyl)
PAG	Periaqueductal grey
PD	Panic disorder
PCR	Polymerase chain reaction
PDSS	Panic Disorder Severity Scale
PET	Positron emission tomography
PSS	Panic Symptom Scale
rCBF	regional cerebral blood flow
SNP	Single nucleotide polymorphism
SPECT	Single photon emission computed tomography
SSRI	Selective serotonin (5-HT) re-uptake inhibitors
TD	Tryptophan depletion
TPH	Tryptophan hydroxylase
VAS	Visual Analogue Scale
5-HT	5-hydroxytryptamine, serotonin
5-HTP	5-hydroxytryptophan
5-HTT	Serotonin (5-HT) transporter

1. INTRODUCTION

1.1. Clinical characteristics of PD

1.1.1. Diagnosis

Panic disorder (PD) is an anxiety disorder characterised by recurrent panic attacks and associated fearful anticipation of panic or its consequences and frequently developing agoraphobia (DSM-IV, ICD-10). Panic attacks are defined as rapidly escalating occurrence of at least four out of 13 distressing symptoms including palpitations, chest pain or discomfort, sweating, shortness of breath, choking, trembling, nausea, dizziness, paresthesias, chills or hot flushes, depersonalisation or derealisation, and fear of dying or losing control. Sporadic panic attacks occur in population at 7–15% lifetime prevalence rate (Pelissolo and Lepine, 1998; Eaton et al., 1994) being often associated with substantial morbidity even in absence of a full clinical manifestation of PD (Klerman et al., 1991). Recognition of PD requires careful differential diagnosis from panic-like symptoms and autonomic arousal resulting from direct physiological effects of substance use or a general medical condition and from symptoms of anxiety better explained by another psychiatric disorder.

1.1.2. Epidemiology

The majority of surveys have found a lifetime prevalence of PD rates between 1.5% and 3.5%, whereas twelve-month prevalence rates are generally about 1% (Eaton et al., 1994; Weissman et al., 1997; Pelissolo and Lepine, 1998). The prevalence of PD is slightly more than twice higher in females than in males (Eaton et al., 1994). The age onset of PD is usually in the mid-twenties, with hazard rates for females ranging from 25 to 35 years and for males between 30 and 45 years (Wittchen and Essau, 1993). Marital status is a significant risk factor for PD: the highest lifetime prevalence rates are found in widowed, separated, or divorced subjects (Wittchen and Essau, 1993). There are no consistent findings concerning the educational level and the risk of developing PD, although Eaton et al. (1994) reported a tenfold higher risk for persons with less than 12 years of education. Other possible risk factors are smoking habits (Pohl et al., 1992; Amering et al., 1999) and existence of pulmonary disease (Zandbergen et al., 1991; Verburg et al., 1995). Several studies have suggested that life events such as early parental loss or childhood abuse may increase the risk of PD (Faravelli, 1985; Tweed et al., 1989).

Patients with PD have an increased risk of other psychiatric as well as medical morbidity. One of the most frequent complications of PD is agoraphobia, which DSM-IV defines as (a) fear of being in places or situations

from which escape might be difficult or help might not be available; (b) these situations are avoided or endured with marked distress or the patient needs a companion; and (c) the fear is not better explained by another mental disorder. The comorbidity of PD with agoraphobia varies from 29.5% to 58.2% (Eaton et al., 1994; Wittchen et al., 1998). There is also a high degree of PD comorbidity with other anxiety disorders: 20–75% for social phobia, 20% for generalized anxiety disorder (GAD), 14% for obsessive-compulsive disorder and 6% for post-traumatic stress disorder (Goisman et al., 1994; Chen and Dilsaver, 1995; Pelissolo and Lepine, 1998). Major depression is the most frequent concurrent diagnosis observed in 30–60% of patients with PD (Weissman et al., 1997; Kessler et al., 1998). Substance abuse is also a common comorbid disorder in up to 36% of cases according to Epidemiologic Catchment Area data (Regier et al., 1990). Most frequently, substance use disorders appear to be secondary to PD and can be interpreted as self-medication (Marshall, 1997; Merikangas et al., 1998). In addition PD often occurs together with cardiac, gastrointestinal, respiratory, and neurological disorders (Zaubler and Katon, 1998).

1.1.3. Implications

PD has been recognised as a chronic condition with fluctuating course (Liebowitz, 1997; Pollack and Otto, 1997). Some studies have addressed longitudinal aspects; for example, in a three-year follow-up only 10% of patients with PD were symptom-free (Noyes et al., 1990) and only 12% of PD patients were in full remission after five years (Faravelli et al., 1995). PD can be a seriously disabling disorder causing impairment in social, personal and occupational functioning and significant loss of quality of life (Candilis et al., 1999). The rate of those PD patients who are dependent on welfare or disability benefits exceeds 25% in some studies (Katerndahl and Realini, 1997).

1.2. Aetiology of PD

1.2.1. Psychological and physiological factors

The pathogenesis of PD is complex involving psychological, biological, and evolutionary factors. According to the cognitive theory of Clark (1988), individuals who experience recurrent panic attacks have a relatively enduring tendency to interpret certain bodily sensations in a catastrophic fashion. Barlow (1988) described panic as the basic emotion of fear, which is considered to be an acute reaction to perceived imminent danger when no danger is present. He identified three types of alarm: true alarms (immediate danger present), false

alarms (panic attacks) and learned alarms (conditioned panic attacks). Barlow's model of PD includes a biological diathesis (propensity to experience arousal under stress) and a psychological vulnerability (influenced by such factors as early life events and parenting style). The false suffocation alarm theory by Klein (1993) suggested that PD patients have a low stimulation threshold of the asphisiostat, a physiological mechanism of protection from potentially lethal stimuli. The anxiety sensitivity theory explains panic phenomena by a belief that beyond any immediate physical discomfort, anxiety, and associated symptoms may cause deleterious physical, psychological, or social consequences (Taylor et al., 1992).

1.2.2. Neuroanatomical basis

Gray (1988) proposed the earliest neuroanatomical model for anxiety. This model outlined a septohippocampal brain circuit and identified behavioural inhibition as one of the potentially important functions for specific brain structures and their connections. More recently, the dorsal periaqueductal grey (PAG), the median hypothalamus, and the amygdala have been implicated in the genesis of panic and anxiety that have been identified during electrical stimulation studies performed during stereotaxic brain surgery (Chapman et al., 1954; Nashold et al., 1969). The PAG has been suggested as the primary coordination site of the all-important defence/escape response to unconditioned aversive stimuli (Bandler and Carrive, 1988; Deakin and Graeff, 1991). Gorman et al. (1989) were the first authors to propose a neuroanatomical model specific to PD. This aetiological model of PD suggested an abnormal sensitivity in the brain mechanisms of fear and alarm response involving a network of neuronal pathways and multiple neurotransmitter systems, such as serotonin (5-hydroxytryptamine, 5-HT), norepinephrine (NOR), gamma-aminobutyric acid (GABA), and others. According to the neuroanatomical hypothesis, panic attacks originate from a dysfunction in the brain fear network that integrates various structures of the brainstem, the amygdala, the medial hypothalamus and cortical regions. This model coherently accounted for the various clinical features of PD, linking panic attacks to discharge from brain stem nuclei, anticipatory anxiety to limbic activation and kindling, and agoraphobia/fearful avoidance to prefrontal cortical function (Gorman et al., 2000). Particular attention has been paid to the amygdala, a phylogenetically ancient structure playing a central role in conditioned fear (Le Doux, 1994). Dysregulation of the amygdala, perhaps due to a deficient control by cerebral structures, may result in amygdaloid activation and panic attack (Grove et al., 1997; Coplan and Lydiard, 1998). The central nucleus of the amygdala is an important site of fear response, with the amygdalofugal pathway projecting from the central nucleus to the PAG, locus coeruleus (LC), the solitary nucleus, the parabrachial nucleus, the nucleus ambiguus, the hypothalamus, and other structures (de Olmos, 1990).

Once activated, the central nucleus of the amygdala is positioned to integrate fear-related responses through a number of different efferents including: (1) activation of the PAG; (2) activation of the noradrenergic cells of LC; (3) activation of the hypothalamic–pituitary–adrenal axis via direct efferents to the paraventricular nucleus and indirect stimulation through the bed nucleus of the stria terminalis, and (4) activation of cardiorespiratory responses through the stimulation of the parabrachial nucleus and the dorsal motor nucleus of the vagus (Davis, 1992). Excitatory projections from the prefrontal cortex may be activated also by panic/anxiety-provoking sensory stimuli or catastrophic cognitions and precipitate panic by activating the basolateral nucleus of the amygdala, which in turn activates the adjacent central nucleus of the amygdala (Amaral et al., 1992). Moreover, retrieval of fear-signifying memories from the adjacent hippocampus may, via reciprocal projections, make an impact on the central nucleus of the amygdala (Amaral and Insausti, 1992).

1.2.3. Challenge studies

Over the past decades one of the main experimental approaches to the neurobiology of PD has been use of provocative agents in laboratory settings. Large number of pharmacological and neurochemical studies confirmed that various agents display panicogenic properties in patients with PD, as well as in patients with other anxiety disorders, and in healthy subjects. The list of the challenge agents includes sodium lactate (Pitts and McClure, 1967), 5% CO2 (Gorman et al., 1984) or 35% CO2 (Griez et al., 1987), yohimbine, an alpha-2 adrenergic antagonist, (Charney et al., 1992), caffeine (Boulenger and Uhde, 1982; Charney et al., 1985), 5-HTergic agents, such as m-chlorophenylpiperazine (m-CPP), a mixed 5-HT agonist-antagonist or fenfluramine, a 5-HT releasing drug (Kahn et al., 1991; Targum and Marshall, 1989), cholecystokinin-tetrapeptide (CCK-4), a CCK receptor agonist (Bradwejn et al., 1991), dopaminergic agents (Pitchot et al., 1992) and opioid agents, such as naltrexone (Maremmani et al., 1998). This variety of implicated mechanisms supports a view that interaction between different neurotransmitters is involved in the pathogenesis of panic attacks and PD (Bourin et al., 1998). The neurotransmitter systems mostly studied in PD are the NORergic, 5-HTergic, CCK and GABAergic ones. The anxiogenic and panicogenic effects of yohimbine and isoproterenol point to an importance of the NORergic system in PD (Pyke and Greenberg, 1986; Charney et al., 1992). Possible NORergic mechanisms include an increased central alpha 2-adrenoceptor sensitivity and hyperactivity of the LC, the main NORergic nucleus (Redmond and Huang, 1979; Nutt, 1986) or an increased firing rate of the LC due to a dysfunction of NORergic receptors (Charney et al., 1992). Experimental and treatment studies also support the involvement of 5-HT system in PD, although the relationship between 5-HT and panic seems to be complex (Bell and Nutt, 1998). With regard to the GABAergic system, a

shift of benzodiazepine receptors to the inverse agonism or relative deficiency of a hypothetical anxiolytic ligand have been proposed to play a role in the pathogenesis of PD (Nutt et al., 1990). The involvement of the CCK system in the neurobiology of PD has been suggested by robust panicogenic properties of CCK-4 in PD patients as well as in healthy subjects (Bradwejn et al., 1991; Shlik et al., 1997a, b). Abnormal hypersensitivity of the CCK2 receptors leading to an excessive stimulation of the nucleus tractus solitarius of the caudal medulla has been postulated to mediate the role of CCK in the pathophysiology of PD (Bradwejn and Koszycki, 1994a). Further, it has been suggested that the ventral medulla possess the requisite "hypersensitive" chemoreceptor apparatus to respond to the metabolic panicogens, such as lactate or CO2 inhalation and hyperventilation (Coplan and Lydiard, 1998).

1.2.4. Brain imaging data

The brain imaging studies have provided further evidence of neuroanatomical substrates involved in PD. The structural brain abnormalities observed in patients with PD include increased ventricle-brain ratios in computed tomography, and focal abnormalities in the temporal area with an increased atrophy in magnetic resonance imaging (MRI) (Fontaine et al., 1990; Vythilingam et al., 2000). Neurophysiological studies have described certain electroencephalographic abnormalities (Stein and Uhde, 1989; Lepola et al., 1990). The positron emission tomography (PET) and the single photon emission computed tomography (SPECT) studies in PD have demonstrated variations of functional activity (regional brain perfusion or metabolism) in the various brain structures, such as parahippocampal gyrus (Reiman, 1987; Nordahl et al., 1998), hippocampus, and parahippocampal area (Bisaga et al., 1998), frontal cortical regions (de Cristofaro et al., 1993; Eren et al., 2003), orbitofrontal, prelimbic, anterior cingulus, parietal, and temporal cortices (Fischer et al., 1998; Meyer et al., 2000). Several SPECT and PET studies showed reduced benzodiazepine receptor binding in various cortical regions in PD patients (Kuikka et al., 1995; Malizia et al., 1998; Bremner et al., 2000).

1.2.5. Genetic factors

The data from twin and family studies strongly indicate the involvement of genetic factors in the susceptibility to PD (Noyes et al., 1986; Kendler et al., 1993; Maier et al., 1993). However, the mode of transmission is unknown and the heritability estimates were in the range of 0.35–0.46, demonstrating equal importance of environmental factors (Kendler et al., 1993). The higher risk of PD in first-degree relatives of PD probands with an earlier onset or with an increased sensitivity to CO2 challenge suggests that the age of onset or response

to panicogenic agents may differentiate familial subtypes of PD (Perna et al., 1996; Goldstein et al., 1997). In line with current neurobiological hypotheses of PD (Bourin et al., 1998) the molecular genetic research have mainly focused on CCK, 5-HT, dopamine (DA), and some other neuroactive systems. On the whole, genetic association studies of PD have provided inconsistent, negative, or not yet replicated results. Conceivably, this is due to the fact that the biology of anxiety disorders involves multiple genes with small effects, their interaction with each other and with nongenetic events (Moldin, 2000). Positive findings were reported on the association between PD with certain polymorphisms of CCK and CCK2 receptor genes (Wang et al., 1998; Kennedy et al., 1999). Further studies of these gene variants have so far produced inconsistent results and no associations were found between CCK1R gene variants and PD (Kato et al., 1996; Kennedy et al., 1999; Hamilton et al., 2001; Hattori et al., 2001; Yamada et al., 2001; Ise et al., 2003). A family-based study focusing on the DA system found no associations between PD and polymorphisms in DA4 receptor and DA transporter genes (Hamilton et al., 2000a). Several studies detected associations between PD and adenosine A2 receptor gene as well as catechol O-methyltransferase gene polymorphisms (Deckert et al., 1998; Woo et al., 2002) while other studies did not support these findings (Ohara et al. 1998; Yamada et al., 2001).

1.3. 5-HT and PD

1.3.1. Brain 5-HT system

5-HT, a heterocyclic (indolic) amine, is a widespread neurotransmitter in mammalian organism, whereas the majority of 5-HT content is presented in enterochromaffin cells of the gastrointestinal tract and cerebral tissue (Kema et al., 2000). 5-HT coexists with galanin, substance P, GABA, and nitric oxide synthase in a significant number of 5-HTergic neurons and interacts with various other neurotransmitter and neuromodulatory systems, including NOR, DA and CCK (Baumgarten and Göthert, 2000).

5-HT function is regulated in multiple steps, such as synthesis, storage, release, and inactivation (Kema et al., 2000). 5-HT is synthesized from the essential amino acid tryptophan via 5-hydroxytryptophan (5-HTP) and stored in reserpine-sensitive vesicles until released into the synaptic cleft by nerve impulses. In the synaptic cleft, 5-HT can act on both postsynaptic receptors that convey information to other neurons and presynaptic autoreceptors that regulate 5-HT synthesis and release. The activation of somatodendritic 5-HT1A auto-receptors decreases 5-HT neuronal firing and, in turn, the synthesis, meta-bolism, and release of 5-HT (Moret and Briley, 1997; Blier et al., 2002).

Similarly, the terminal 5-HT1B/D autoreceptors control the amount of 5-HT released from the neuron terminal (Middlemiss et al., 1988; Maura et al., 1993). Recently it has been shown that the functional activity of the presynaptic 5-HT1B autoreceptor can be specifically modulated by an endogenous tetrapeptide, 5-HT-moduline (Massot et al., 1996). It has been proposed that the binding of 5-HT-moduline to 5-HT1B receptors leads to the inactivation of these receptors (Grimaldi and Fillion, 2000). Additionally, 5-HT release may be indirectly stimulated by 5-HT3 and 5-HT4 receptors and regulated by receptors of other non-5-HTergic systems, such as GABA, NOR, CCK (Baumgarten and Göthert, 2000). Inactivation of 5-HT occurs mainly with reuptake by a membrane carrier, 5-HT transporter (5-HTT), which removes 5-HT from the synaptic cleft back into the neuron (Lesch and Mössner, 1998). Intraneuronal breakdown is accomplished via oxidative deamination by the mitochondrial flavoprotein monoamine oxidase (MAO), resulting in the metabolite 5-hydroxyindolacetic acid. At least two forms of this enzyme, MAO-A and MAO-B are known. MAO-A has the highest affinity for 5-HT and NOR and is expressed at the highest level in catecholaminergic neurons (Fowler et al., 1987; Thrope et al., 1987). MAO-B is expressed at highest levels in astrocytes and 5-HTergic neurons and is more active in deaminating benzylamine and phenylethylamine (Thrope et al., 1987; Weyler et al., 1990).

Anatomically, the central 5-HT system consists of groups of neurons whose cell bodies are located in the brainstem and raphe nuclei and complex axonal systems, which innervate receiving areas throughout the nervous system from the spinal cord to the cortex (Jacobs and Azmitia, 1992; Lucki, 1998). There are six ascending pathways originating from 5-HT raphe nuclei, two of which pass through the medial forebrain bundle. Pontine and medullary nuclei project caudally to the spinal cord, where 5-HT is involved in pain perception, visceral regulation, and motor control. Midbrain nuclei, particularly the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN), innervate the forebrain and are likely to modulate cognitive, affective, and neuroendocrine functions (Azmitia and Whitaker, 1995). Among the limbic forebrain areas, the septum and the dorsal hippocampus are innervated mainly by the MRN whereas the amygdala and the ventral hippocampus receive 5-HT input almost exclusively from the DRN. The hypothalamus is innervated by both nuclei, but these projections are segregated: the DRN innervates mainly the medial and the MRN innervates the lateral hypothalamus. The fibres arriving at the periventricular hypothalamus come via the dorsal raphe-periventricular tract, a fibre system that also projects to the thalamus and the PAG matter of the midbrain. Most of the 5-HTergic projections to the basal ganglia, substantia nigra and LC arise from the DRN. The 5-HT projections to the neocortex are highly branched and terminate in all cortical layers. The majority of them come from the DRN through the dorsal raphe-cortical tract, which projects diffusely to the parietal and temporal cortices. There is also a ventral projection to the piriform and entorhinal cortices via the dorsal raphe-forebrain tract. The cortical projections

of the MRN also travel via the medial forebrain bundle and reach the frontal, cingulated, and entorhinal cortices (Graeff, 1997). Morphologically, the efferent fibres of the DRN and MRN differ from each other, suggesting the possibility of a dual 5-HTergic system. The fibres of DRN are fine axons with small varicosities, whereas the thicker fibres originating from the MRN are beaded with large spherical varicosities (Törk and Hornung, 1990). Also, the terminals from the DRN establish preferential contact with 5-HT2A/2C receptors, whereas those from the MRN relate to postsynaptic 5-HT1A receptors (Mamounas et al., 1991).

Due to high bifurcation and arborescence of 5-HT system, 5-HT is released from nerve terminals in virtually all brain regions, which explains the involvement of 5-HT in a broad range of functions of the central nervous system (CNS). The research has shown that 5-HT plays an important role in neuronal development, thermoregulation, pain sensitivity, motor regulation, sleep–wake cycle, appetite, sexual behaviour, aggression, learning, and memory. Furthermore, the 5-HT system has been implicated in the pathophysiology of neurodegenerative and psychiatric disorders, such as Alzheimer's disease, schizophrenia, depression, GAD, PD, and obsessive-compulsive disorder, as well as in the mode of action of psychotropic drugs (Lucki, 1998). The multiple effects of 5-HT in CNS are mediated by several 5-HT receptors, classified into seven main groups, comprising a total of 14 structurally, genetically, and pharmacologically distinct 5-HT receptor subtypes (Zifa and Fillion, 1993). It has been established that 5-HT receptor families (5-HT1 to 5-HT7) are mostly seven putative transmembrane spanning, G-protein coupled receptors with the exception of the 5-HT3 family, which belongs to the ligand-gated ion channel (Maricq et al., 1991). The 5-HTergic G-protein coupled receptors have been shown to either inhibit the activity of adenylyl cyclase, decreasing the production of adenosine 3′,5′-cyclic monophosphate (5-HT1 family) or activating it (5-HT4, 5-HT6, 5-HT7), or stimulating the phosphoinositides metabolism (5-HT2 family). Furthermore, it has been proposed that these receptors can be coupled to various G-proteins depending on the tissue or cells (Dickenson and Hill, 1998) and can crosstalk with other G-protein coupled receptors (Selbie and Hill, 1998). Various 5-HT receptor subtypes are known to mediate a wide range of neurochemical and neuroendocrine responses. Activation of 5-HT receptors increases the release of NOR (5-HT1A), DA (5-HT3, 4 indirectly), acetylcholine (5-HT1A), GABA (5-HT3) and CCK (5-HT3). On the other hand the activation of these 5-HT receptors decreases the release of NOR (5-HT2A, 2C), DA (5-HT2C) acetylcholine (5-HT1B, HT3, HT4) and glutamate (5-HT1A). Activation of 5-HT receptors also increases secretion of the adrenocorticotropic hormone (5-HT1A, 2A, 2C, 3), cortisol (5-HT1A, 2A, 3, 4), prolactin (5-HT1A, 2A, 2C, 3), renin (5-HT2A, 2C) and oxytocin (5-HT2A, 2C) (Baumgarten and Göthert, 2000).

1.3.2. 5-HT and the neurobiology of PD

The 5-HT system has previously been implicated in PD both directly and indirectly (Coplan et al., 1992). Evidence for primary 5-HTergic dysregulation in patients with PD has accumulated from a number of studies comparing patients to normal subjects. For instance, PD patients manifest significant differences in 5-HTergic function from healthy controls, including (1) reduced 5-HT platelet transporter sites (Faludi et al., 1994; Marazziti et al., 1999); (2) heightened neuroendocrine responses to 5-HT agonists (Kahn et al., 1988a; Targum and Marshall, 1988; Apostolopoulos et al., 1993); and (3) anxiogenic responses to certain direct and indirect 5-HT agonists, such as *d*-fenfluramine (dFEN), m-CPP, and clomipramine (Kahn et al., 1988b; Targum and Marshall, 1988; Klein et al., 1991; George et al., 1995; Meyer et al., 2000).

Two opposing hypotheses have been put forward to explain PD by 5-HTergic dysfunction: 5-HT excess or overactivity (Iversen, 1984) and 5-HT deficit or underactivity (Deakin and Graeff, 1991; Eriksson, 1993). The 5-HT excess theory suggests that patients with PD either have an increased level of 5-HT release or a hypersensitivity of postsynaptic 5-HT receptors. The 5-HT deficit theory proposes that in particular brain regions 5-HT has a restraining effect on panic behaviour. In case of 5-HT deficit this restraint is reduced and panic ensues. The main evidence for the overactivity of 5-HT in PD comes from challenge studies, where 5-HT agonists, such as m-CPP and releasing agents, such as dFEN, acutely induce panic and/or anxiety symptoms in PD patients (Kahn et al., 1988b; Targum and Marshall, 1989). It was shown also that the PD group had an augmented cortisol release induced by oral administration of m-CPP (0.25 mg/kg) when compared to the control or groups of depressive patients (Kahn et al., 1988a). However, in another study Kahn et al. (1991) found that oral administration of m-CPP (0.25 mg/kg) induced augmented adrenocorticotropic hormone and prolactin release in PD females but not in PD males. Subsequent studies by the Yale group, with a lower intravenous m-CPP dose, yielded more panic effects in patients with PD and a blunted prolactin response in female patients (Germine et al., 1994). These results suggested possible gender differences in m-CPP neuroendocrine responses and hypersensitivity of 5-HT receptors. Additionally, behavioural hypersensitivity to the oral administration of m-CPP and selective 5-HT1A agonist, ipsapirone, has been previously demonstrated in PD patients, suggesting opposite changes in the responsiveness of 5-HT2C and 5-HT1A-related receptors in PD patients (Broocks et al., 2000). Clinically, the selective 5-HT re-uptake inhibitors (SSRI) induced behavioural hypersensitivity, which PD patients almost invariably manifest at the onset of treatment, has been postulated to be the result of up-regulation of 5-HTergic postsynaptic receptors (Coplan et al., 1992). Nevertheless the view of supersensitive postsynaptic 5-HT receptors has not always been supported. There is a number of negative studies resulting in lack of conclusive evidence for postsynaptic receptor hypersensitivity (Charney et al., 1987;

Wetzler et al., 1996). Studies using 5-HT precursors, tryptophan or 5-HTP have not demonstrated any differences between the reactivity of PD patients and healthy control subjects (Charney and Heninger, 1986; den Boer and Westenberg, 1990a). In addition, studies using the intravenous m-CPP did not initially reveal biochemical or behavioural differences between PD and healthy subjects (Charney et al., 1987; Wetzler et al., 1996). Finally, the fact that 5-HT antagonists have no clinically relevant anti-panic effects argues against the 5-HT overactivity theory of PD (den Boer and Westenberg, 1990b; Marek et al., 1992). The 5-HT deficit theory proposes that 5-HT has a restraining effect on panic. Clinical and challenge studies provide support to this hypothesis. Treatment studies consistently show efficacy of medications increasing the synaptic availability of 5-HT, such as SSRIs and MAO inhibitors, in the treatment of PD (Tyrer and Shawcross, 1988; Kent et al., 1998; Nutt, 1998). Experimental studies have demonstrated that lowering of 5-HT levels by tryptophan depletion (TD) resulted in an increased sensitivity to panicogenic effects of flumazenil and 35% CO2 (Klaassen et al., 1998; Schruers et al., 2000; Bell et al., 2002). An earlier study in healthy volunteers indicated that TD did not influence the panicogenic effect of CCK-4 although it did augment CCK-4-mediated neuroendocrine activation (Koszycki et al., 1996). Conversely, the administration of 5-HTP restrains panic response to 35% CO2 challenge in patients with PD (Schruers et al., 2002). There is also an earlier observation that patients with PD felt relief from the effect of 5-HTP (den Boer and Westenberg, 1990a). The administration of dFEN has been reported to inhibit 7% CO2 panic challenge and had a beneficial effect in the treatment of PD (Solyom, 1994; Mortimore and Anderson, 2000). Additionally, it has been demonstrated that treatment with SSRIs significantly decreases the sensitivity of PD patients to CCK-4, flumazenil, and CO2 panicogenic effects (Bertani et al., 1997; Perna et al., 1997; Shlik et al., 1997c; van Megen et al., 1997; Bell et al., 2002).

To explain the controversial function of 5-HT in anxiety and panic responses, Deakin and Graeff (1991) hypothesized that the 5-HT system plays a dual role in the modulation of different forms of pathological anxiety. They proposed that panic attacks are caused by a spontaneous neuronal discharge in the PAG and that direct 5-HTergic projections from the DRN to dorsal PAG inhibit this activity. Thus, the PAG-originated discharge is more likely to occur if the 5-HT restraining mechanism is impaired. In addition, experiments with microinjection of drugs directly into the PAG indicated that 5-HT1A and 5-HT2A receptors inhibit defensive behaviour and aversion generated in the PAG (Graeff, 1993). On the other hand, according to the Deakin–Graeff theory, 5-HT projections facilitate conditioned responses to aversive stimuli, as well as anticipatory or generalized anxiety, at the level of the temporal lobe structures and the amygdala. Furthermore, the central nucleus of the amygdala may play a role rather in anticipatory anxiety than in spontaneous panic (Graeff, 1990). However, the ventral amygdalofugal pathway projects to the PAG, both directly and indirectly (via the paraventricular nucleus), implicating 5-HT-induced

amygdaloid hyperactivity in the mediation of anticipatory anxiety as well as induction of the priming process of the PAG, lowering its threshold for the occurrence of panic (Grove et al., 1997). It has been suggested that in PD, normally anxiogenic stimuli may become panicogenic due to loss of normal DRN-mediated suppression of the PAG and/or due to a failure of the MRN to contain chronic stress-induced overstimulation of the central nucleus of the amygdala/PAG axis. Also, 5-HT-induced enhancement of the MRN function could suppress cortical and amygdalo-hippocampal activation while enhancement of the DRN function could restore inhibitory restraint over the PAG and dampen the positive feedback loop via cortical and thalamic connections into the panic neurocircuitry (Grove et al., 1997). In addition to the inhibitory effect of 5-HTergic neurones on sensory input to the amygdala (Stutzmann and LeDoux, 1999) they have an inhibitory effect on NORergic neurones in the LC (Törk and Hornung, 1990). Therefore, 5-HT deficit would be expected to result in increased NORergic activity and subsequent autonomic symptoms of panic. 5-HT system may mediate inhibitory effects not only at the level of LC but also at several important NORergic projection sites in limbic structures (amygdala, dorsal hippocampus) and NORergic brain stem nuclei that modulate cardio-respiratory functions (Coplan and Lydiard, 1998). Furthermore, evidence suggests that enhanced 5-HTergic activity results in reduced release of the anxiogenic neurotransmitter corticotropin-releasing factor from the hypothalamus (Kent et al., 1998).

1.3.3. Genetic regulation of 5-HT and PD

There is an increasing interest in molecular genetic research aiming to detect associations between PD and putative vulnerability genes of the 5-HT system. However, the 5-HT gene polymorphisms responsible for the biological basis of PD have not yet been established. Previously no associations were found between PD and the polymorphisms in tryptophan hydroxylase (TPH), 5-HTT, 5-HTR1A, 5-HTR1B and 5-HTR2C genes (Deckert et al., 1997; Han et al., 1999; Deckert et al., 2000; Fehr et al., 2000 a, b; Fehr et al., 2001; Inada et al., 2003) although positive associations have been observed between PD and polymorphisms in the MAO-A gene and the 5-HTR2A gene (Deckert et al., 1999; Inada et al., 2003).

2. RATIONALE FOR THE STUDIES

The complex involvement of 5-HT in anxiety and panic requires further research. To continue testing some aspects of the above-mentioned hypotheses we have performed series of studies on 5-HT function in PD using experimentally induced panic attacks as well as methods of brain imaging and genotyping. An important premise for a better understanding of 5-HT involvement in PD is the necessity to investigate the 5-HT modulatory role under conditions of acute panic symptomatology. Furthermore, it is not clear as yet whether the immediate influence of 5-HT on panicogenesis has the same anti-panic effects as chronic treatment with 5-HT-enhancement antidepressants. Thus, the experimental panic challenge tests with approaches to manipulate the 5-HT system could clarify the impact of 5-HT on the pathophysiology of panic attacks and PD. In our first direction of research we hypothesised that the administration of 5-HT immediate precursor 5-HTP would lead to an increased presynaptic availability of 5-HT and thereby reduce the susceptibility to panic attacks. To test this hypothesis we investigated the effect of acute 5-HT-enhancement with 5-HTP on the panicogenic properties of CCK-4 challenge in healthy subjects taking into account possible influence of the 5-HT-related gene variants. Another important target of research in this area is the function of 5-HTT that plays a pivotal role in fine-tuning of 5-HT neurotransmission as well as in the mechanism of action of SSRIs. The evaluation of functional activity of 5-HTT may be accessible using neuroradiological detection of ligand binding properties. To investigate this aspect we conducted two controlled studies using the SPECT technology to detect quantitative alterations in 5-HT re-uptake sites in the brain regions that are involved in the neurobiology of anxiety in patients with PD and GAD. Particularly, we aimed to detect whether the 5-HTT binding characteristics in patients with PD are related to their symptomatic status and whether the role of 5-HTT in PD differs from its role in GAD. The third research question that we attempted to address was the role of genetic regulation of 5-HT in the predisposition to PD. Additional knowledge on this may be achieved by association studies of functionally relevant candidate genes related to the 5-HT system as well as using technological advances in the molecular genetics allowing simultaneous detection of multiple single nucleotide polymorphisms (SNPs) and haplotypic association. To pursue these directions we performed two studies comparing the genotype distributions in patients with PD and healthy subjects with the focus on 5-HT related genes.

3. AIMS OF THE STUDIES

The general objective of the present work was to study the role of the 5-HT system in the pathogenesis and neurobiology of PD, embracing a broad scope of investigations from experimental studies to brain imaging techniques and genetics. The specific aims were as follows:

1. To investigate the influence of 5-HT-enhancement with 5-HTP on the panicogenic properties of CCK-4 challenge in healthy subjects (I).
2. To examine the influence of functional polymorphisms in 5-HT-related genes on the CCK-4-induced panic rate in healthy subjects (II).
3. To evaluate the brain 5-HTT binding potential in patients with PD and GAD in comparison to healthy subjects using the SPECT methodology (III, IV).
4. To detect possible associations between polymorphisms in the genes related to the 5-HT system and PD phenotypes (V, VI).

4. MATERIALS AND METHODS

4.1. Subjects

All studies were conducted at the Department of Psychiatry of the University of Tartu (Tartu, Estonia). The imaging procedures of Study III were carried out at the Department of Clinical Physiology and Nuclear Medicine of Kuopio University Hospital in Kuopio, Finland.

The subjects in Studies I and II were 32 healthy volunteers (14 males and 18 females; mean age 22.4 years, range 18–32 years) recruited by flyer advertisement. The inclusion criteria were as follows: no personal or family psychiatric history and good physical health as evidenced by medical history and physical examination. None of the subjects in these studies had a history of psychiatric disorders, panic attack, or substance abuse as assessed by the Mini International Neuropsychiatric Interview M.I.N.I. 5.0.0. (Sheehan et al., 1998) or a first-degree relative with a history of mental illness. None of the volunteers had a positive urine test for benzodiazepines, cocaine, amphetamines, hallucinogens, opioids, or cannabis at the time of the screening visit. All participants were required to abstain from alcohol for at least two weeks before the study. Lack of recent alcohol use was confirmed during the interview and physical examination. Pregnancy was excluded in all females by a urine pregnancy test, and females were studied within the first week of their menstrual cycle.

The subjects in Studies III and IV were 8 patients (1 male and 7 females; mean age 34.8 years) with current PD, 8 patients (1 male and 7 female; mean age 32.0 years) with PD in remission and 7 (4 male and 3 females; mean age 39.3 years) patients with GAD selected among the outpatients at the Clinic of Psychiatry of the Tartu University Clinics and by newspaper advertisement in Tartu, Estonia. The matched healthy control subjects in these studies were recruited by newspaper advertisement in Kuopio, Finland (Study III) or in Tartu, Estonia (Study IV). The diagnosis of PD and GAD according to DSM-IV criteria was verified using the M.I.N.I. interview and substantiated by psychiatric history and medical records. The additional inclusion criteria for patients were: right-handedness, good physical health, not pregnant, absence of other comorbid psychiatric disorders, free of alcohol and benzodiazepines for at least 2 weeks before the imaging procedures. None of the patients had received any antidepressant or other medication known to affect the 5-HTT binding for at least 4 months before the study.

The participants in Study V (Table 1) consisted of 158 patients with PD (80% females, mean age 38.0 years) with or without agoraphobia recruited at the Clinic of Psychiatry of Tartu University Clinics and 215 healthy subjects (74% females, mean age 39.8 years) with no personal or family history of psychiatric disorders among first-degree relatives recruited by a newspaper

advertisement in Tartu, Estonia. The sample of Study VI (Table 2) consisted of 127 patients with PD (82% females, mean age 38.4 years) with or without agoraphobia recruited at the Clinic of Psychiatry of Tartu University Clinics and 146 healthy subjects (75% females, mean age 38.7 years) with no personal or family history of psychiatric disorders among first-degree relatives recruited by a newspaper advertisement in Tartu, Estonia. The diagnosis of PD according to DSM-IV criteria was verified using M.I.N.I. interview and substantiated by psychiatric history and medical records. The PD patients with comorbid unipolar or bipolar major depression or other anxiety disorders were included in the study, but no other psychiatric comorbidity was allowed. The patients and healthy volunteers in Studies V and VI were unrelated individuals of Caucasian origin. The majority of the subjects among patients and controls were ethnic Estonians.

4.2. Ethical considerations

The Human Studies Ethics Committee of the University of Tartu approved the study protocols and the informed consent forms, and all participants provided written informed consent. The institutional ethics committee at the Kuopio University Hospital approved the protocol of Study III.

4.3. Challenge substances and procedure

CCK-4 (tryptophan-methionine-aspartic acid-phenylalanine-NH_2) was purchased from Clinalfa AG (Läufelfingen, Switzerland) in vials containing 50 μg of the substance. 5-HTP was purchased from Sigma-Tau (Rome, Italy) as a medication TRIPT-OH in 100 mg capsules. The placebo capsules were prepared at the Department of Pharmacy of the University of Tartu. Study I was conducted in a double blind placebo-controlled parallel group design. An equal number of males and females were randomized to receive either 200 mg of 5-HTP or placebo in analogous capsules. For CCK-4 injection an intravenous catheter was inserted into an antecubital vein and a physiological saline drip was started. CCK-4 was injected 90 minutes after administration of 5-HTP or placebo in a dose of 50 μg in 2.5 ml of normal saline solution as a bolus push.

4.4. Behavioural and cardiovascular assessments

The panic response to CCK-4 was evaluated by the Panic Symptom Scale (PSS) based on the DSM criteria for panic attack (Bradwejn et al., 1991). The subjects rated the intensity of 18 panic symptoms on a scale from 0 – not present to 4 – extremely severe. The main outcome measure was occurrence of the panic attack that was a priori defined as presence of four or more symptoms with a sudden onset and at least moderate severity plus "anxiety/apprehension/fear" item with a score 3 or 4. Other measures derived from the PSS were the sum intensity score, defined as the sum of all individual item ratings and subscale scores for somatic (items 1–10, 12–14) and cognitive symptoms (items 11, 15–18). The visual Analogue Scale (VAS) on a 100 mm line (most relaxed to most anxious) was used as a measure of subjective anxiety at baseline and thereafter at 30, 60, and 90 minutes prior to the injection of CCK-4. Within 5 minutes after CCK-4 injection the participants rated the level of anxiety that they experienced on the peak of the CCK-4-induced symptoms on the VAS and the peak intensity of the CCK-4-induced symptoms on the PSS. Arterial blood pressure and heart rate were recorded at baseline and thereafter at 30, 60, and 90 minutes prior to the injection of CCK-4 by an automatic monitor Lohmeier (B. Braun Melsungen AG, Germany). The blood pressure and heart rate were measured every 30 seconds during the first two minutes and 5 minutes after the CCK-4 injection.

4.5. Clinical assessments

In Study III prior to the start of brain imaging procedures the patients were evaluated by the Panic Disorder Severity Scale (PDSS), the score ranging from 0 to 28 (Shear et al., 2001), and on the Hamilton Anxiety Scale (HAMA) ranging from 0 to 42 (Hamilton, 1959). Prior to the first scan the patients rated the VAS, ranging from 0–100 mm (most relaxed to most anxious).

In Study IV prior to the start of brain imaging procedures patients were assessed for the symptoms of anxiety with the HAMA, and for symptoms of depression on the Montgomery-Åsberg Depression Rating Scale ranging from 0 to 60. Prior to the first scan the patients and healthy subjects rated their anxiety on a VAS.

4.6. Imaging procedure and data processing

Iodine-123 labelled 2 ß-carbomethoxy-3β-(4-iodophenyl) or [^{123}I] nor-ß-CIT was used as a radioligand in Studies III and IV. Nor-ß-CIT is an analogue of previously used ß-CIT with a high affinity to both 5-HTT and DA transporter, and a higher affinity to 5-HTT in comparison with ß-CIT with well characterized kinetics (Bergström et al., 1997; Hiltunen et al., 1998). A single dose of 185 MBq of [^{123}I] nor-ß-CIT (supplied by MAP Medical Oy, Tikkakoski, Finland) was diluted in a volume of 10 ml physiological saline and slowly injected into the right antecubital vein in a dark and quiet imaging room. Whole-head serial scans (5 min, 6 hours, 24 hours after injecting the tracer) were performed on a Siemens Multi SPECT 3 gamma camera with fan-beam collimators (Study III) and on a two-head General Electric 2000 gamma camera (Study IV). The radius of rotation was less than 14 cm. Scan was acquired for an angular step of 3° over 180°/camera head. The total scanning time was 30 min. The SPECT scans were decay-corrected and reconstructed with Butterworth-filtered back projection in a 128x128 matrix with a pixel size of 3x3 mm, and were attenuated-corrected by a Chang's algorithm (Hiltunen et al., 1998; Kuikka et al., 1993). The imaging resolution was 8–9 mm (in Study III) and 13–15 mm (in Study IV). The SPECT slices were summarized to the total slice thickness of 6 mm and realigned along with the lines of Talairach coordinates using a semiautomatic brain quantification program of the HERMES software (Nuclear Diagnostics AB, Stockholm, Sweden). Regions of interest included the midbrain, the thalamus, the temporal cortex, the anterior cingulum, and the cerebellum as a reference region. The regions of interest were manually positioned with the help of the laboratory's own 5-HTT based control template. The specific binding of nor-ß-CIT to 5-HTT in a given region was calculated using a reference region model. The main assumptions of this model are that the distribution volume of nonspecifically bound ligand is the same for both target and reference tissues and that the delivery of the tracer from arterial blood is the same in both regions (Acton et al., 1999). A graphical method (Logan et al., 1990) was applied to estimate the specific binding (= distribution volume ratio, V_D) from the slope of the time-activity curves. The analyses were done blindly without knowing the anamnestic data of the subjects. To test the reproducibility of our ROI and analysing method, the whole data sets of index subjects were re-analysed within 3 months interval by the same analyser. The intraclass correlation coefficient was used. In Study III the intraclass correlation coefficient was 0.89 for the thalamus, 0.86 for the midbrain, 0.77 for the right temporal cortex, 0.69 for the left temporal cortex and 0.60 for the anterior cingulus, respectively. In Study IV the intraclass correlation coefficient was 0.67 for the midbrain, 0.63 for the thalamus, and less than 0.60 for the temporal lobes and the anterior cingulus, respectively

4.7. DNA analysis

In Studies II and V, DNA was extracted from 5 ml of venous blood using standard phenol-chloroform extraction. The polymorphisms were genotyped according to previously described protocols (Lappalainen et al., 1995; Battersby et al., 1996; Lesch et al., 1996; Nielsen et al., 1997; Sabol et al., 1998). In brief, for genotyping deletion/insertion polymorphism in the promoter of 5-HTT gene, the region was amplified using primers HTT-LPR-A (5'- GGC GTT GCC GCT CTG AAT GC) and HTT-LPR-B (5'-GAG GGA CTG AGC TGG ACA ACC AC). Polymerase chain reaction (PCR) products were resolved by electrophoresis in a 2% agarose gel. The HTT VNTR polymorphism was genotyped using PCR with oligonucleotides HTT-VNTR-A (5'-GTC AGT ATC ACA GGC TGC GAG) and HTT-VNTR-B (5'-TGT TCC TAG TCT TAC GCC AGT G). DNA fragments were run in a non-denaturing polyacrylamide gel electrophoresis and stained by ethidium bromide. To genotype an adenosine-cytosine transversion at nucleotide 218 in intron 7 of TPH gene, the intron was amplified using primers 5'-TTC AGA TCC CTT CTA TAC CCC AGA and 5'-GGA CAT GAC CTA AGA GTT CAT GGC A. Amplified DNA fragments were digested overnight at 37°C with 4 U of NheI and separated by electrophoresis in a 1.8% agarose gel. MAO-A promoter region polymorphisms were identified using PCR with primers MAOaPB1 (GAA CGG ACG CTC CAT TCG GA) and MAOaPT1 (ACA GCC TGA CCG TGG AGA AG), followed by electrophoresis in polyacrylamide gel and staining with ethidium bromide. The HT1B receptor 861G/C polymorphism was genotyped using PCR with oligodeoxynucleotide primers 5'-GAA-ACA-GAC-GCC-CAA-CAG-GAC-3' (sense), 5'-CCA-GAA-ACC-GCG-AAA-GAA-GAT-3' (antisense). The HT1B amplicons were digested overnight with HincII and DNA fragments separated using agarose gel electrophoresis.

In Study VI genomic DNA was isolated from peripheral blood leukocytes using standard high-salt extraction techniques. Amplification of genomic fragments containing the studied polymorphic sites was performed in 64 individual PCR reactions (single or multiplex) using standard or touchdown conditions. A 20% fraction of the dTTP in the amplification mixture was substituted by dUTP allowing later fragmentation of PCR products with uracil-*N*-glycosylase. Pooled amplification products were concentrated and purified, followed by fragmentation and functional inactivation of the unincorporated dNTPs. The production of oligonucleotide microchips and Arrayed Primer Extension (APEX) reactions were performed as described earlier (Tõnisson et al., 2002). The polymorphisms were identified by Genorama™ 4.1 genotyping software by using signal patterns from a wild-type DNA sequence as the reference. The association analysis included 90 polymorphisms selected from 21 candidate genes including 86 SNPs and 4 insertions/deletions (Study VI,

Table 1). Table 1a presents also the description of selected from the 5-HT system SNPs analysed in Study VI.

Table 1a. Description of selected SNPs from the 5-HT system

Gene and SNP	Position from ATG	Location	Allele 1	Allele 2
5-HTR1A –1018	5-HTR1A -1019	5q11.2-q13	C	G
5-HTR1A –480	5-HTR1A –480	5q11.2-q13	A	del
5-HTR1B	5-HTR1B -1089	6q13	T	C
5-HTR1B	5-HTR1B -700	6q13	C	A
5-HTR1B -511	5-HTR1B –511	6q13	G	T
5-HTR1B -161	5-HTR1B –161	6q13	A	T
5-HTR1B 129	5-HTR1B 129	6q13	C	T
5-HTR1B 276	5-HTR1B 276	6q13	G	A
5-HTR1B 371	5-HTR1B 371	6q13	T	G
5-HTR1B 705	5-HTR1B 705	6q13	C	T
5-HTR1B 861	5-HTR1B 861	6q13	G	C
5-HTR1B	5-HTR1B 1180	6q13	G	A
5-HTR2A -1438	5-HTR2A –1437	13q14-q21	A	G
5-HTR2A 73	5-HTR2A 74	13q14-q21	C	A
5-HTR2A 102	5-HTR2A 102	13q14-q21	T	C
5-HTR2A 1354	5-HTR2A 61008	13q14-q21	C	T
5-HTR2C 68	5-HTR2C 4390	Xq24	G	C
5-HTR2C 2831	5-HTR2C 181359	Xq24	T	G
5-HTR3A 1302	5-HTR3A -507	11q23.1-q23.2	T	C
5-HTR3A 1596	5-HT3A 14378	11q23.1-q23.2	G	A
5-HTT	5-HTT 18784	17q11.1-q12	A	C
5-HTT	5-HTT 10647	17q11.1-q12	G	A
5-HTT	5-HTT 167	17q11.1-q12	G	C
TPH1 218	TPH1 14494	11p15.3-p14	A	C
TPH1 779	TPH1 15055	11p15.3-p14	A	C

4.8. Statistical analysis

The data were analysed using the software package STATISTICA 5.1 (StatSoft Inc, Tulsa, OK, USA, 1997). The Wilcoxon signed-rank test and Mann-Whitney U-test were used to analyse the non-parametric data. The proportions were

compared by Pearson Chi-squire tests. One-way analysis of variance (ANOVA) was used to compare the cardiovascular responses to CCK-4 injection between 5-HTP and placebo groups (Study I) and to compare the binding data and clinical characteristics between the groups (Studies III and IV). Correlations were estimated by Pearson product-moment correlation analysis. The data are presented as mean values and standard deviation (SD). The genotype and allele frequencies between the patient and control groups in Studies II and V were compared by the chi-square (χ^2) test using the software package STATISTICA 5.1. Odds ratio (OR) values and 95% confidence intervals (CI) were calculated using STATA 6.0. The association analyses in Study V were done for all patients (n=158), and separately for subgroups of agoraphobic patients (n=87) and without agoraphobia (n=71). The results were considered as suggestively significant at the level of $p<0.05$ with a conservative estimation of significance after correction for multiple comparisons on 4 genes at the level of $p<0.0125$. The association analysis in Study VI was performed using GENEPOP Version 3.3 software (Raymond and Rousset, 1995). The comparisons of allele frequencies in this study were done between the patients and controls with separate analyses for the PD-pure and PD-comorbid subgroups. Haplotype analysis was performed using the maximum likelihood method for estimating simultaneously haplotype frequencies and haplotype-phenotype association as described elsewhere (Tregouet et al., 2002). Pairwise LD were estimated by a log-linear model and the extent of disequilibrium was expressed as standardized D` characteristic. Only genes genotyped for two or more SNPs and showing the presence of LD in both the affected and control group and having preliminary evidence of marker-disease association were included in the haplotype analysis. To reconstruct accurate multimarker haplotypes, the markers used for further analyses were tested for Hardy-Weinberg equilibrium and for having no Mendelian inheritance errors. Genotypes were obtained for 98% of the samples tested. Several SNP markers showing association with PD were not included in the haplotype analysis due to a very low allelic frequency in the affected group. Using the inference method, it was also possible to investigate the effect of each locus on different haplotypic backgrounds. The odds ratio for PD was estimated according to the haplotypic background conferred by other polymorphisms. The level of statistical significance was set at $p=0.05$ for nominal association without correction for multiple comparison due to the exploratory character of Study VI.

5. RESULTS AND DISCUSSION

5.1. Study I: The effect of 5-HTP on CCK-4-induced panic attacks

5.1.1. Panic and anxiety symptoms

Panic attacks were observed in 3 subjects in the 5-HTP group and in 7 subjects in the placebo group with a difference not reaching the statistical significance (χ^2=2.3, df=1, p=0.13; Figure 1). On the PSS there were trends for a lower sum intensity in the 5-HTP group with no significant difference in the somatic or cognitive subscale scores (Table 1). The data on the individual panic symptoms showed that choking was of a significantly less intensity after 5-HTP compared with placebo (1.38 ± 1.26 vs. 2.56 ± 1.09; U=61.0, p=0.01), and a similar trend was observed for abdominal distress (1.69 ± 1.40 vs. 2.63 ± 1.41; U=80.5, p=0.07). On the VAS there was a significant increase in the anxiety ratings after CCK-4 injection in both the 5-HTP and the placebo groups with no between-group differences in baseline, peak or net increase anxiety (Table 1).

5.1.2. Gender differences

The panic rate was lower in females after 5-HTP compared to the placebo (n=1, 11.1% and n=6, 66.7%; χ^2=5.8, df=1, p=0.016) and equally low in both groups in males 2 (28.6%) and 1 (14.3%), respectively (Figure 1). There was a trend towards a significant decrease in the somatic but not cognitive symptoms in male subjects after 5-HTP (p=0.06). On the contrary, the cognitive but not somatic symptoms were significantly lower in females after 5-HTP comparing to the placebo group (p=0.03; Table 1). In comparison with the placebo the administration of 5-HTP significantly lowered the intensity of the symptom of choking in females (1.33 ± 1.23 vs. 2.89 ± 0.93; U=14.0, p=0.02) as well as intensity of the symptom of hot flashes /cold chills in males (1.29 ± 0.95 vs. 2.71 ± 1.38; U=8.0, p=0.04).

5.1.3. Cardiovascular response

The groups did not differ in baseline values of the heart rate or blood pressure before receiving 5-HTP or placebo or before the CCK-4 challenge (Table 1). The heart rate increased significantly after CCK-4 injection in 5-HTP from 65.2 ± 10.7 to 89.4 ± 19.2 (Z=3.5, p=0.0004) and in the placebo group from 62.6 ± 8.0 to 86.1 ± 18.1 (Z=3.5, p=0.0004). Systolic blood pressure increased significantly after CCK-4 injection in 5-HTP from 125.8 ± 12.7 to 140.4 ± 14.4

(Z=2.8, p=0.005) and in the placebo group from 126.3 ± 11.4 to 131.5 ± 14.5 (Z=2.1, p=0.04). Also diastolic blood pressure increased significantly after CCK-4 injection in 5-HTP from 76.2 ± 7.0 to 84.9 ± 8.4 (Z=3.0, p=0.003) and in the placebo group from 76.4 ± 7.8 to 79.9 ± 8.5 (Z=2.4, p=0.02). The increase in the heart rate was somewhat higher at the peak of CCK-4 effects in those who received 5-HTP than in the placebo group (74.4 ± 14.7 vs. 66.0 ± 8.7; F(1.30)=3.7, p=0.07). Also, there were significantly higher increases in blood pressure at the peak of CCK-4 effects in 5-HTP group compared to the placebo: for the systolic blood pressure 140.4 ± 7.5 vs. 128.5 ± 11.2; F(1.30)=12.6, p=0.001; and for the diastolic blood pressure 87.2 ± 5.5 vs. 80.1 ± 5.8; F(1.30)=12.7, p=0.001; respectively).

5.1.4. Discussion

The main finding of this study is a significant reduction in the rate of panic attacks and in the intensity of cognitive symptoms following CCK-4 injection by acute pre-treatment with 5-HTP in female healthy subjects. The effect of 5-HTP on CCK-4-induced panic in male volunteers was limited to lowering the intensity of somatic symptoms. This is in accordance with a recent finding by Klaassen et al. (1998) which showed that TD significantly increased the neurovegetative but not cognitive panic symptoms induced by CO2 in healthy male subjects. In another study, however, TD failed to modify the panicogenic effects of CCK-4 in healthy males (Koszycki et al., 1996). Thus the results of our study suggest the presence of gender differences in the 5-HT-ergic influence on the CCK-4-induced panic and/or in 5-HT-CCK interaction. A previous brain imaging study established a lower brain 5-HT metabolism in healthy females compared to males (Nishizawa et al., 1997). On the other hand, the greater sensitivity to CCK-4 effects in females was demonstrated in current sample and has also been previously reported in patients with PD (Ströhle et al., 2001). Therefore, an enhanced 5-HT availability after receiving 5-HTP may have a more obvious inhibitory influence on the CCK-4-induced panic in female subjects. However, the nature of interaction between 5-HT and CCK-4 effects is not clearly established as yet. It has been proposed that CCK-4 induces somatic panic symptoms by a direct action on the brainstem nuclei and the cognitive symptoms by excitation of other neurotransmitter systems projecting from the brainstem to the limbic and cortical areas (Bradwejn and Koszycki, 1994a). It has also been suggested that CCK-4-induced panic attack in humans should ultimately involve the PAG as a coordinative region in the mediation of panic (Mongeau and Marsden, 1997). Thus, an increased availability of 5-HT may restrain CCK-4-induced panic via inhibitory influence on these pathways. Previously it has been demonstrated that treatment with SSRIs significantly decreases the sensitivity of PD patients to CCK-4 effects (Shlik et al., 1997c; van Megen et al., 1997). Studies with ondansetron, an antagonist of 5-HT3

receptor, have shown its efficacy in attenuating CCK-4-induced panic in healthy male volunteers acutely but not after chronic treatment (Depot et al., 1998). Furthermore, pre-treatment with ondansetron did not prevent the panicogenic effects of CCK-4 analogue pentagastrin in patients with PD (McCann et al., 1997). It is conceivable that 5-HT influences the CCK-ergic effects also indirectly via other neurotransmitter systems.

This study did not find significant differences between the effects of 5-HTP and placebo on CCK-4-induced anxiety when rated on VAS. A previous study by Klaassen et al. (1998) also did not find differences between TD and placebo on the VAS-anxiety ratings after CO2 challenge in healthy subjects. They suggested that the healthy subjects might have experienced a negative affect that they did not equate with anxiety. However, a significant difference in VAS-rated anxiety between TD and placebo was observed in patients with PD (Schruers et al., 2000). Thus, it is possible that different estimation of anxiety by non-panickers and panickers in this study has led to this confusion. It is also conceivable that the effects of 5-HTP may have more specifically an antipanic than a generally anxiolytic character.

As in the previous studies (Shlik et al., 1997b) it was found that CCK-4 caused significant increases in blood pressure and the heart rate as well as breathing difficulties. The cardiorespiratory effects of CCK-4 are probably mediated via the nucleus tractus solitarius, which relays viscerosensory information to the parabrachial nucleus and promotes cardiorespiratory and other autonomic responses via medullary and spinal cord efferent pathways (Coplan and Lydiard, 1998). The 5-HT-ergic neurons in the MRN exert modulatory effects on these brainstem nuclei controlling cardiovascular and respiratory functions (Blier et al., 1987; Grove et al., 1997). Furthermore, 5-HT may have a tonic inhibitory influence on the respiratory hyperactivity accompanying the panic attack (Kent et al., 1996). Accordingly, in this study the subjects who received 5-HTP reported significantly less CCK-4-induced symptoms of choking. An intriguing finding was a significantly greater increase in blood pressure after CCK-4 injection in 5-HTP-group in comparison to the placebo. A similar trend was observed for the heart rate. Although it is known that 5-HT has vasotonic properties, the mechanism of these paradoxical cardiovascular effects of 5-HTP during CCK-4-induced panic is not clear. Interestingly, chronic treatment with imipramine antagonized increases in systolic and diastolic blood pressure as well as in the heart rate induced by CCK-4, although the treatment with citalopram did not substantially influence the cardiovascular responses to CCK-4 in PD patients (Bradwejn and Koszycki, 1994b; Shlik et al., 1997c). The differences between acute and chronic influences of 5-HT on the panic responses as well as the distinct pharmacological profiles of imipramine, citalopram, and 5-HTP may account for these contradictions.

There have been a number of studies on the acute influence of manipulations with the 5-HT system in the laboratory models of panic attacks. Usually the findings in healthy volunteers have been more modest than in patients with PD.

For instance, Schruers et al. (2002) did not find any significant influence of acute administration of 5-HTP on the CO2-induced panic in healthy volunteers although they found a significant reduction in panic by 5-HTP in patients with PD. Similarly, the augmenting effects of TD on the CO2 panic have been more significant in patients with PD than in healthy subjects (Klaassen et al., 1998; Schruers et al., 2000). Nevertheless, the panicogenic effects of CCK-4 in our study as assessed by VAS or PSS appear to be more pronounced than in the healthy volunteers in studies with CO2-induced panic (Klaassen et al., 1998; Schruers et al., 2000, 2002) probably due to a greater panicogenic/anxiogenic potency of CCK-4.

The limitation of this study is a relatively small sample size. As this study was not specifically planned to investigate the effects of gender, these effects should be examined in a sufficiently powered study. In sum, the results of this study suggest gender-dependent influences of the acute oral administration of 5-HTP on CCK-4-induced panic. The increased 5-HT availability significantly reduced the rate of CCK-4-induced panic attacks and cognitive symptoms in females and lowered the somatic panic symptoms in male healthy subjects. These results provide further support to the hypothesis of a specific inhibitory influence of 5-HT on panic attacks (Deakin and Graeff, 1991).

5.2. Study II: The influence of functional polymorphisms in 5-HT-related genes on the CCK-4-induced panic rate

5.2.1. Distribution of genotypes

The distribution of genotypes in the total sample for 5-HTTLPR was the following: LL 31% (n=10), LS 56% (n=18) and SS 13% (n=4); and for MAO-A: 1–(1) 34% (n=11), 3–1 22% (n=7) 3–(3) 38% (n=12) and 4–3 6% (n=2). There was no significant difference in the distribution of the genotype frequencies between males and females. Distribution of the genotypes in females was similar in 5-HTP and placebo groups. In male subject distributions of 5-HTTLPR but not of MAO-A genotypes significantly differed between 5-HTP and placebo groups (χ^2=7.37, df=2, p=0.03).

5.2.2. Associations between 5-HT-related genotypes and CCK-4-induced panic

For further analysis genotypes were organized in two groups similarly to previous studies (Lesch et al., 1996; Deckert et al., 1999). The 5-HTTLPR alleles were combined in one group with all S allele carriers (SS and SL

genotypes) and the other group included only LL-homozygous. The MAO-A alleles were divided in a group with all carriers of shorter alleles (1–(1) and 1–3 genotypes) and a group of carriers of longer alleles (all subjects with 3–(3) and 3–4 genotypes). Table 1 presents the associations between genotype groups and the rate of CCK-4-induced panic attacks. In the total sample the presence of MAO-A polymorphisms with longer alleles was associated with a lower panic rate (p=0.01). No associations were found between the panic rate and genotypes of 5-HTTLPR (p=0.12). The following analysis by sex showed lack of significant associations between MAO-A or 5-HTTLPR polymorphisms and the panic rate in males. In females the panic rate was significantly lower in the group with longer alleles of MAO-A polymorphism (p=0.007) as well as in the group with S alleles of 5-HTTLPR (p=0.03). Additionally, the panic rate was significantly lower in females with longer alleles of MAO-A polymorphism who received placebo but did not differ in the 5-HTP pretreatment group (χ^2 =5.14, df=1, p=0.02 and χ^2 =1.41, df=1, p=0.24, respectively). On the contrary, the panic rate in females was significantly lower in the group with 5-HTTLPR S alleles after 5-HTP but not with placebo pretreatment (χ^2 =3.94, df=1, p=0.047 and χ^2 =2.25, df=1, p=0.13 respectively). In the total sample and individually in males there were no associations between candidate polymorphisms and the rate of panic response separately in 5-HTP and the placebo groups.

5.2.3. Discussion

The current study showed a lower frequency of CCK-4-induced panic attacks in healthy subjects with high activity MAO-A gene allelic variants. In addition, the CCK-4-induced panic rate was significantly lower in the healthy females with high activity MAO-A alleles or with short allele of the 5-HTTLPR compared to females with low activity MAO-A alleles or with LL-genotype of the 5-HTTLPR. Thus, the panic rate was affected by genetic polymorphisms with identified functional activity and, therefore, apparently related to the regulation of 5-HT neurotransmission. Interpretation of these findings is confounded by the use of 5-HTP pretreatment in half of the subjects, which in own turn reduced the panic rate in female subjects. Conceivably, the effects of 5-HTP and functional 5-HT-related gene polymorphisms may have additive antipanic properties. Nevertheless, the association between high activity MAO-A polymorphism and reduced panic attacks in females contradicts the hypothesis of the inhibitory effect of increased 5-HT availability on panicogenesis. A possible explanation of these contradictory results is related to different roles of 5-HT in the modulation of panic responses on the level of 5-HT-ergic neuronal cell bodies in the midbrain region and on the level of the 5-HT-ergic neuronal terminals in other brain regions. Perhaps an increased availability of 5-HT in the midbrain may have likewise antipanic effects as decreased 5-HT tone in the 5-

HT-ergic projections to other brain region. On the other hand, MAO-A enzyme is know to be expressed at the highest level in catecholaminergic neurons and is more active in deaminating both 5-HT and NOR (Fowler et al., 1987; Thrope et al., 1987). Thus, in addition to the 5-HT-related mechanisms, an increased activity of MAO-A enzyme could have antipanic effects due to increased inactivation of NOR resulting in a lower NORergic tone in the panic-related brain structures. However, previously a significant association was found between PD and high activity MAO-A gene polymorphism in female patients (Deckert et al., 1999). This relationship differs from the finding of this study of a protective effect of this polymorphism in CCK-4 challenge, which may indicate a distinct genetic background of panic attacks in patients with PD and experimental panic attacks in healthy subjects. The complexity of PD phenotype, which in addition to panic attacks, includes anticipatory and phobic anxiety, may account for these contradictions, whereas MAO-A gene may have a different influence on various components of PD (Deckert et al., 1999).

The previous genetic studies failed to prove an association between a functional polymorphism in the 5-HTT-gene promoter region and PD (Deckert et al., 1997; Hamilton et al., 1999; Ishiguro et al., 1997). However, Hamilton et al. (1999) have reported a more frequent occurrence of 5-HTT LL genotype in female PD probands compared to female controls. Furthermore, our recent association study in PD patients revealed significantly higher frequencies of 5-HTTLPR long allele as well as LL genotype in patients than in healthy subjects. These findings are in line with present results showing that females with the LL genotype are more sensitive to CCK-4 panic compared to S allele genotype and suggest a possible role of high-expressive 5-HTTLPR long allele in predisposition to PD. Interestingly, that Schmidt et al. (2000) reported a significant difference in fearful response to CO2 inhalation between 5-HTTLPR genotype groups in healthy subjects. In their sample, subjects homozygous for the long variant (L) appeared to be at risk for behavioral hyperreactivity to a challenge with 35% CO2. However, in patients with PD, CO2 reactivity was not influenced by 5-HTTLPR genetic variants (Perna et al., 2004). Nevertheless, in healthy volunteers the short allele of 5-HTTLPR has been associated with anxiety-related personality traits (Lesch et al., 1996) and with greater activation of the amygdala in response to fearful face stimuli (Hariri et al., 2002). Thus, the low-expressive 5-HTTLPR short allele may be related to anxiety proneness, which contradicts the antipanic direction of this genotype in this study. This discrepancy may be explained by different roles of the 5-HT system in the neuronal circuits of anxiety and panic attacks according to the theory by Deakin and Graeff (1991).

This study showed that neither MAO-A nor 5-HTTLPR gene variants affected the frequency of panic attacks in male subjects. This is in line with evidence that acute administration of 5-HTP did not decrease the rate of CCK-4 induced panic attacks in healthy males. A likely reason for this failure to demonstrate antipanic effects of 5-HTP and 5-HT-ergic genes polymorphisms is

the low number of CCK-4 panic attacks in males in the current study. However, sex-related differences in the genetic background of 5-HT (Deckert et al., 1999; Hamilton et al., 1999) as well as in the susceptibility to panic attacks should be taken into account.

The major limitations of the current study in respect to the genetic findings include concurrent use of 5-HTP and the small sample size, which may have led to false positive results. The study was primarily designed to test putative antipanic properties of 5-HTP. However, the detection of genotype influences in the secondary analysis underlines the importance of incorporating factors of genetic variability in this type of studies. Further investigations in larger samples are needed for the validation of the current findings and for drawing more reliable conclusions. The role of 5-HT-related genetic variants requires more extensive investigation in healthy subjects and in patients with PD and other anxiety disorders. Use of panic challenge with CCK-4 or other appropriate laboratory agents could help to uncover vulnerability to panic and relate it to a genetic disposition to panic attacks and PD.

5.3. Study III: SPECT study of 5-HTT binding in PD

5.3.1. Brain perfusion and 5-HTT binding

The analysis of the first SPECT scan (5 min) did not show any significant differences in the perfusion of the regions of interest between the three groups. The volume of distribution (V_D) demonstrated a significant difference in the [^{123}I] nor-ß-CIT binding to 5-HTT between the patients and controls (Table 2). The patients with current PD had significantly lower 5-HTT binding in the midbrain raphe region than healthy controls or the patients with PD in remission (Figures 1 and 2). The binding of 5-HTT did not differ between the patients in remission and healthy subjects. In addition, the patients with current PD had significantly lower 5-HTT binding in the temporal lobes as well as in the thalamus by comparison with the controls. The 5-HTT binding in the temporal cortex in the patients in remission was between the binding indices of the patients with current PD and controls without significant between-group differences. However, similarly to the patients with current PD, the patients in remission had a marked reduction in the thalamic 5-HTT binding in comparison to controls.

5.3.2. Correlation between scales and 5-HTT binding

The correlation analysis in the separate patients groups did not show any significant associations between the PDSS, HAMA, or VAS scores and binding characteristics in the brain regions of interest. However, in the total sample of patients (n=16) the PDSS score was significantly and inversely correlated with the 5-HTT binding in the midbrain raphe as well as in both temporal lobes (Table 3, Figure 3). The binding to the midbrain 5-HTT was also inversely correlated with the HAMA and VAS scores (Table 3).

5.3.3. Discussion

The results of this study show that the patients with current PD have significantly lower 5-HTT binding in the midbrain raphe, in the temporal lobes, and in the thalamus than the healthy control subjects. The patients with PD in remission, on the contrary, have normal 5-HTT binding properties in the midbrain and in the temporal regions but a significantly lower thalamic 5-HTT binding. Furthermore, there are significant inverse correlations between the severity of PD symptoms rated on the PDSS and the midbrain, the temporal, but not the thalamic 5-HTT binding in the total sample of patients. These findings point to a dysregulation in the 5-HT system with an altered function of 5-HTT related to the clinical status of patients with PD.

There is strong evidence that a deficit in the brain 5-HT raises but an increase reduces the susceptibility to panic attacks (Klaassen et al., 1998; Schruers et al., 2000, 2002; Bell et al., 2002). Thus, a low brain 5-HTT density in the symptomatic patients with PD may mark a decreased 5-HT function. Functionally, a decrease in the brain 5-HTT binding might reflect a compensatory process in the 5-HT system attempting to increase the concentration of synaptic 5-HT and, thus, to prevent the panic attacks. In other words, the possible shortage in the availability of 5-HT in panic patients may be a factor initiating the adaptive processes leading to a decrease in the expression of the brain 5-HTT. Such an adaptive change in the expression of 5-HTT is supported by results of animal studies showing a significant reduction in the expression of brain 5-HTT after a decrease in 5-HT induced with TD (Linnet et al., 1995; Ramos et al., 2000). Concurrently, there was recently showed that clinical improvement in obese binge-eating females after chronic treatment with an SSRI and group psychotherapy resulted in a significant increase in their brain 5-HTT binding/density (Tammela et al., 2003). Perhaps an increase in the brain 5-HTT binding after successful treatment in these patients occurred in connection with the rise in the availability of brain 5-HT. On the other hand, the reduction in the brain 5-HTT in panic patients appears to lie on the same continuum with the action mechanism of SSRIs in the treatment of PD. Hence, if SSRIs exert their therapeutic effects in PD by increasing the synaptic

availability of 5-HT (Nutt et al., 1999), then a decrease in the brain 5-HTT expression in PD patients could be a putative endogenous mechanism for the achievement of the same effects. An alternative explanation for the decreased 5-HTT density is possible rarefaction of 5-HT neurons in patients with PD. However, this would contradict the findings of normal 5-HTT density in remitted patients, and there are no data on neuronal loss in PD.

In regard to the temporal lobes, the previous studies of patients with PD have shown alterations in the regional cerebral blood flow (rCBF) and in the metabolism as well as a lower density of benzodiazepine receptors in this region (Schlegel et al., 1994; Bisaga et al., 1998; Meyer et al., 2000; Boshuisen et al., 2002). In addition, PD patients also revealed qualitative and quantitative abnormalities of the volume of the temporal lobes (Ontiveros et al., 1989; Fontaine et al., 1990; Vythilingam et al., 2000). Perhaps the smaller volume of the temporal lobes in PD patients is one reason for their decreased temporal 5-HTT binding/density. Although the patients in remission did not significantly differ from the patients with current PD in the temporal 5-HTT binding, their binding characteristics were closer to healthy controls than to the patients with current PD. This suggests that, similarly to the midbrain, the temporal 5-HTT binding normalizes in the remitted PD patients along with the clinical improvement. Recently Meyer et al. (2000) have found that PD females have significantly decreased rCBF in the left posterior parietal–superior temporal cortex in comparison to healthy females. However intravenous administration of dFEN induced a significantly greater rCBF increase in the same region in PD female than in controls. The authors concluded that the rCBF change may be a direct consequence of the release of 5-HT, whereas tonically decreased release of 5-HT in this region could create a low activation at the baseline, and an increased sensitivity to dFEN -induced 5-HT release (Meyer et al., 2000). However, the previous study did not find any the difference in rCBF in the left posterior parietal–superior temporal cortex between depressed and healthy subjects neither before nor after intravenous administration of dFEN (Meyer et al., 1998). Moreover, the decreased left inferior parietal cortex metabolism has been reported to normalize in imipramine-treated PD subjects (Nordahl et al., 1998).

In comparison to healthy subjects there was a significant decrease in the thalamic 5-HTT binding in both patients with current and remitted PD. This suggests that a decrease in the thalamic 5-HTT binding may be a trait marker independent of the symptomatic status. However, a relatively small sample size may have prevented the detection of a statistically significant difference in the thalamic 5-HTT binding between groups. Considering the role of the thalamus in the brain anxiety circuitry (Gorman et al., 2000), a decreased thalamic density of 5-HTT may reflect an increased interoceptive sensitivity and anticipatory anxiety, which is common for the patients with PD.

Recently, using PET Neumeister et al. (2004) found a marked reduction of cerebral 5-HT1A receptors binding in the anterior and posterior cingulate

cortices, and the midbrain raphe in patients with PD without any between-group differences in the anterior insula, mesiotemporal cortex, and the anterior temporal cortex. Another PET study demonstrated that untreated PD patients have a reduced binding to 5-HT1A receptors in raphe region as well as in the amygdala, orbitofrontal and temporal cortices. However fully recovered PD patients after treatment with SSRIs showed a normalized density in the postsynaptic receptors but an unaffected reduction in the raphe receptors (Nutt et al., 2003). Activation of somatodendritic 5-HT1A autoreceptors causes a reduction in the firing rate of 5-HTergic neurons and leads to the suppression of 5-HT synthesis, 5-HT turnover, and 5-HT release in diverse projection areas (Moret and Briley, 1997). In contrast, when acting directly at postsynaptic 5-HT1A receptors in target areas, agonists mimic the effect of 5-HT released, thereby facilitating 5-HT neurotransmission (Lanfumey and Hamon, 2000). Thus, the reduced 5-HT1A density may be similar to the reduction of 5-HTT compensatory importance for an increase in synaptic 5-HT availability. Nevertheless, postsynaptic 5-HT1A receptor down-regulation is probably not expected to reflect a compensatory response to abnormal 5-HT release because reducing 5-HT transmission by lesioning the raphe or administering 5-HT synthesis inhibitors does not alter 5-HT1A receptor binding in the cerebral cortex or the hippocampus (Verge et al., 1986; Frazer and Hensler, 1990; Hensler et al., 1991; Pranzatelli, 1994). Increased 5-HT transmission via chronic administration of SSRI or MAOI antidepressant drugs does not consistently alter 5-HT1A receptor density or mRNA concentrations in the cortex, hippocampus, or amygdala (Welner et al., 1989; Hensler et al., 1991; Spurlock et al., 1994; Carli et al., 1996). Additionally, in experimental animals the genetic expression of 5-HT1A is decreased by the glucocorticoid hormone secretion associated with repeated stress (Lopez et al., 1999). However, glucocorticoid (i.e. cortisol) secretion is generally not elevated in PD and does not increase during panic attacks (Charney and Drevets, 2002).

Nevertheless the reduction of both pre- and postsynaptic 5-HT1A receptors as well as 5-HTT binding, was also previously reported in depressive disorders (Malison et al., 1998; Drevets et al., 2000; Sargent et al., 2000; Willeit et al., 2000). The finding of reduced 5-HTT and 5-HT1A receptor binding in PD and depression could be construed as support for the large twin literature, suggesting that depression and anxiety disorders have important genetic overlaps (Kendler et al., 2003). Also, these findings are in good accordance with evidence of the 5-HT deficit function in both PD and depression (Maes and Meltzer, 1995; Graeff et al., 1996).

Several limitations of our study should be taken into account. First, the sample included only a few male patients. There is evidence of a gender difference in the 5-HT synthesis in healthy subjects (Nishizawa et al., 1997), and PD is more prevalent among females. Therefore, male and female PD patients may differ in their 5-HTT binding potential as well as in the role of 5-HTT in the neurobiology of PD. The second limitation is a relatively high level

of anxiety in patients before the first scan. We cannot exclude the possibility that the state anxiety immediately before the imaging procedure influenced the 5-HTT binding findings. Also, the absence of MRI co-registration may be a technical limitation to confirming some findings of this study.

In conclusion, the current study demonstrates that the patients with current PD have a significantly decreased 5-HTT binding in the midbrain, in the temporal lobes and in the thalamus. The patients with PD in remission have normal 5-HTT binding in the midbrain and in the temporal regions but a significant decrease in the thalamic 5-HTT. The reduced density of 5-HTT in the brain of patients with PD may reflect a deficit of neuronal 5-HT or a compensatory process in the 5-HT system attempting to increase the availability of synaptic 5-HT. Clinical improvement in the patients in remission is related to the normalization of the 5-HTT binding in the midbrain and in the temporal regions whereas a reduced 5-HTT in the thalamus may serve as a marker of interoceptive sensitivity and anticipatory anxiety.

5.4. Study IV: SPECT study of 5-HTT binding in GAD

5.4.1. Brain perfusion and 5-HTT binding

The analysis of the first SPECT scan (5 min) demonstrated a significantly higher perfusion in the midbrain in patients than in healthy subjects (81.4 [10.2] vs. 66.7 [11.0]; $F_{1.12}$=6.76 p=0.02). Additionally the patients had significantly higher perfusion in the cerebellum (73.7 [7.9] vs. 58.3 [10.1]; $F_{1.12}$=10.05 p=0.008).

The distribution volume ratios show that there were no significant differences in the [^{123}I]nor-ß-CIT binding to 5-HTT in the midbrain or in the thalamus between the patients and the controls (Table 1). The female patients had a numerically lower 5-HTT binding in the midbrain raphe region than healthy female controls (0.63 [0.10] vs. 0.71 [0.10]; $F_{1.4}$=1.12 p=0.35) while the male patients had a numerically higher 5-HTT binding in the midbrain raphe region than healthy males (0.73 [0.10] vs. 0.61 [0.10]; $F_{1.4}$=2.79 p=0.15). However, these differences did not reach the level of statistical significance.

5.4.2. Correlation between scales and 5-HTT binding

The correlation analysis in the patient group showed a significant and negative association between the VAS score of anxiety immediately before the first scan and midbrain 5-HTT binding (r = –0.79, p = 0.035; Figure 2). This correlation was not present in the controls or in the total group or with thalamic 5-HTT

binding. There was no correlation between HAMA scores and midbrain or thalamic 5-HTT binding characteristics in the patients.

5.4.3. Discussion

The present study did not find any differences in the binding properties of brain 5-HTT between the patients with GAD and healthy subjects. This may indicate that the functional activity of 5-HTT is intact in GAD. However, the study has several limitations to be considered when interpreting the results. The 5-HTT binding properties, especially in the midbrain, were unusually low in all subjects. This was likely due to a relatively weak resolution of the SPECT camera used in the study. A small sample size could be another possible reason for negative findings. The power analysis showed that the study was only adequately powered (0.8) to detect a 33% difference in 5-HTT binding in the thalamus and a 28% difference for the midbrain. This suggests that if there was a difference in 5-HTT binding between patients with GAD and healthy controls it was likely to be less than 30%. Nevertheless, the results of the present study are in contrast to our previous finding in PD and may imply a different functional role of 5-HTT in GAD than in PD. Moreover, the number of pharmacological studies have also demonstrated different response patterns in patients with GAD and PD to 5-HTergic stimulation. The administration of the nonselective 5-HT1 and 5-HT2 receptor agonist m-CPP led to an increased anxiety and hostility in patients with GAD as well as with PD although its true panicogenic effect is not clearly established (Charney et al., 1987; Germine et al., 1992). Additionally, the 5-HT uptake blocker and 5-HT releasing agent dFEN tends to increase generalized or anticipatory anxiety rather than induce the panic attacks (Graeff et al., 1996; Mortimore and Anderson, 2000). Buspirone is a partial 5-HT1A agonist, which is primarily works presynaptically on the 5-HT1A autoreceptors and, therefore, inhibits the firing of 5-HT cells. It is effective in the treatment of GAD, but its therapeutic efficacy in PD is not supported (Bell and Nutt, 1998). Nevertheless, at higher doses buspirone may also work postsynaptically and, hence, increase panic and anxiety (Frazer and Lapierre, 1987). There is also some evidence that other selective 5-HT1A receptor agonist, such as ipsapirone, might be effective in the treatment of GAD (Cutler et al., 1993). However, acute administration of ipsapirone may be followed by panic attacks in patients with PD (Broocks et al., 2003). The patients with PD did not respond clinically to 5-HT2A receptor antagonists, such as ritanserin, which actually exacerbate some symptoms although they may have some beneficial effects in the treatment of GAD (Bressa et al., 1987; Den Boer and Westenberg, 1990b). Furthermore, the treatment with SSRIs may be accompanied by exacerbation of the anxiety symptoms in the patients during the initial phases of treatment. However, an increase in the frequency of panic attacks is unusual (den Boer and Westenberg, 1990b; Bell and Nutt, 1998).

Thus, these data are in accordance with the hypothesis by Deakin and Graeff (1991), which suggest that the 5-HT system in anxiety disorders has a dual role with an excess activity in GAD and deficit in PD.

Similarly to our previous study in PD, we found that the state of anxiety immediately before the start of imaging procedure may have influenced the end results of the 5-HTT binding properties, at least in the midbrain. There was a significant and inverse correlation between VAS scores and midbrain 5-HTT binding in the patients but not in controls or in the total sample. The patients were also somewhat more anxious before the first scan and had significantly higher perfusion in the studied brain regions than the control subjects. The association between the midbrain 5-HTT binding and the state of anxiety in patients may indicate an increased sensitivity of the midbrain 5-HT system to anxious stimuli. This inverse correlation may also reflect the displacement of radioligand binding to 5-HTT by an increased synaptic 5-HT concentration in raphe which may accompany the rise in anxiety.

In conclusion, the results of this study failed to demonstrate a robust alteration in the functional activity of 5-HTT in patients with GAD. However, the study lacked sufficient power to detect the possible smaller 5-HTT binding differences between the patients and the controls. Further imaging studies in larger samples are needed to validate the present results and to draw more reliable conclusions.

5.5. Study V: Associations between 5-HT related gene polymorphisms and PD

5.5.1. 5-HTTLPR and PD phenotypes

Significant differences were found in the distribution of 5-HTTLPR genotypes and allele frequencies between patients and controls with the LL genotype and L allele variant being more frequent in patients (Table 2). The analysis in subgroups indicated that this difference was significant for both PD subgroups, except for the non-significant difference in genotypic frequency for PD patients with agoraphobia as compared to controls (Table 2). Although the distributions of 5-HTTLPR genotypes for the whole PD group, PD with agoraphobia group, and controls were in agreement with the Hardy-Weinberg equilibrium (p=0.26, 0.53 and 0.82, respectively), the frequency of this genotype significantly deviated from the Hardy-Weinberg equilibrium in the subgroup of PD without agoraphobia (p=0.02). Additionally, the comparison according to the functional classification of Lesch et al (1996), LL genotype versus SS and SL genotypes, indicated significant differences in genotypic frequencies between total PD group and controls (χ^2 =3.95, df=1, p=0.047; OR=1.53; CI 95%=1.01–2.31) as

well as between PD with the agoraphobia group and controls (χ^2 =4.57, df=1, p=0.03; OR=2.25; CI 95%=1.33–3.81).

5.5.2. MAO-A promoter region variants and PD phenotypes

The comparisons of MAO-A promoter region variants between the studied groups were made according to the functional classification of Sabol et al (1998). There were no significant differences in genotype or allele frequencies of MAO-A promoter region polymorphism between the patients and the controls (Table 3). A separate analysis of these polymorphisms in females did not show any significant difference in allele frequencies of MAO-A promoter region polymorphisms between the PD groups and the controls (Table 4). However, a significantly higher frequency of functionally more active genotypes (3–3 or 2–3) of MAO-A promoter region was observed in PD female patients with agoraphobia but not in other female PD groups (Table 4).

5.5.3. Other 5-HT-related gene polymorphisms and PD phenotypes

Finally, no differences were found between the patients and controls in allele distributions of 5-HTT VNTR, TPH 218A/C or 5-HT1B receptor 861G/C polymorphisms for any of the studied groups (Table 5). Also, the genotypic distribution of these polymorphisms did not significantly differ between any of the studied patient groups and controls (data not shown). The distribution of genotypes of these polymorphisms did not deviate from the Hardy-Weinberg equilibrium either in patient groups or in controls.
After correction for multiple comparisons on 4 genes, only the finding of a higher frequency of L allele in 5-HTTLPR in patients remained statistically significant ($p<0.0125$).

5.5.4. Discussion

5-HTT determines the magnitude and duration of postsynaptic receptor-mediated signalling, thus playing a pivotal role in the fine-tuning of 5-HT neurotransmission (Lesch and Mössner, 1998). A number of studies have attempted to find associations between 5-HTTLPR variants and psychiatric disorders (Glatt and Freimer, 2002). The results of previous case-control and family-based association studies argued against the major role of 5-HTTLPR in PD (Deckert et al., 1997; Ishiguro et al., 1997; Matsushita et al., 1997; Hamilton et al., 1999). However, Hamilton et al. (1999) have detected a more frequent occurrence of 5-HTTLPR LL genotype in female PD probands compared to female controls. This finding is in line with the present results showing an

association between L allele as well as LL genotype and PD. Thus, 5-HTTLPR LL genotype that is related to the higher reuptake of 5-HT may be a factor in the predisposition to PD. However, the history or concurrent comorbidity with mood disorders in the sample of this study confounds the role of 5-HTTLPR long allele genotype in PD. Considering that previous association studies in mood disorders have shown either lack of association with 5-HTTLPR variants (Rees et al., 1997; Minov et al., 2001) or positive association with short, but not long alleles of 5-HTTLPR (Collier et al., 1996; Bellivier et al., 1998; Hauser et al., 2003), it is unlikely that our findings could be attributed to the comorbidity with mood disorders. Interestingly, the presence of the long allele of 5-HTTLPR was found to be more frequent in excessively shy children (Arbelle et al., 2003) and in patients with the obsessive-compulsive disorder (Bengel et al., 1999), implicating the role of this variant in the development of some type of anxiety.

A recent family-based study did not find any associations between PD and MAO-A promoter polymorphism (Hamilton et al., 2000b). Nevertheless, similarly to the previous finding by Deckert et al. (1999) we found a significant excess of functionally more active MAO-A promoter alleles in females with PD with agoraphobia than in female controls. However this association was not observed in the total group of PD females. Recently, Inada et al. (2003) have found a significant association with 5-HT2A receptor gene 102T/C polymorphism in PD patients with agoraphobia, but this association was not detected in the PD subgroup without agoraphobia. Also, in a recent case-control study, Rothe et al. (2004) found a significant association between 5-HTR1A receptor –1018C-G polymorphism and PD comorbid with agoraphobia, but not with the total sample of PD patients. These findings are in line with the data of Noyes et al. (1986) suggesting that PD with agoraphobia is a more severe phenotype of PD with a stronger genetic component.

In conclusion, an over-representation of the high-expressing variant of 5-HTTLPR was detected in patients with PD and a significantly higher frequency of the transcriptionally more active MAO-A promoter polymorphism in PD females with agoraphobia. On the other hand, the non-functional serotonergic polymorphisms 5-HTT VNTR, TPH 218A/C and 5-HT1B receptor 861G/C were not associated with PD, which probably reflects lack of influence of these polymorphisms on 5-HT neurotransmission. Interestingly, some recent studies have suggested that a newly identified TPH gene isoform 2 (TPH2), rather than TPH1, is preferentially expressed in the neuronal tissue and has functional polymorphisms involved in the regulation of the brain 5-HT synthesis (Zhang et al., 2004). These data indicate the importance of inclusion of TPH2 polymorphisms in further genetic association studies in anxiety disorders.

It should be noted that after correction for multiple comparisons only results on significance level $p<0.0125$ should be considered as significant. Therefore, the probability of false positive findings on significance level $p<0.05$ due to type I error could not be excluded. Another limitation of our study is a relative excess of female subjects. A reason of this disproportion seemed to be the

exclusion of male PD patients with concurrent alcohol use disorders from our study. However this also reflects higher rates of PD and probably a better response to recruitment in females. Other limitations of this study are the relatively small sample size and significant comorbidity with mood disorders. Thus, additional larger studies are needed to validate and understand the association between 5-HTT and MAO-A functional polymorphisms and PD.

5.6. Study VI: Association study of 90 candidate gene polymorphisms in PD

5.6.1. Association Analysis

In a screening set of 90 polymorphisms 8 SNP markers in 8 genes displayed at least a nominal association with any of the studied PD subgroups (Table 3). The PD-pure phenotype was associated with the markers –94G-A in DA1 receptor gene and 102T-C in 5-HT2A receptor gene. For 102T-C, a significant excess of allele 102C was present in the affected group. On the contrary, the DA1 receptor allele –94A was more frequent in control subjects. These two SNPs appeared to be specific for PD-pure phenotype as no associations were observed in PD-comorbid or in the PD-all group. The PD-comorbid phenotype was distinctively associated with CCK1 receptor 246G-A polymorphism. Associations were also observed between both PD-comorbid and PD-all phenotypes and markers 1270C-G in CCK gene, and –1018C-G in 5-HTR1A gene. CCK marker had a higher 1270G allele frequency in affected subjects, whereas 5-HTR1A allele –1018G was present in a higher proportion of control individuals. For the whole group associations were found for CCK2 receptor marker –215C-A, 5-HTR2C missense SNP 68G-C (Ser23Gly) and –1217Gdel polymorphism in DA4 receptor gene. All these SNPs had higher minor allele frequencies in control subjects. No significant associations were observed between any of the 24 endogenous opioid system-related polymorphisms in five different genes and PD phenotypes.

5.6.2. Haplotype Analysis

Haplotype analysis was performed in the whole data set (cases + controls, n=273). Haplotypes for PD-all group were constructed from SNPs in two genes, CCK and DA1 receptor. Additional haplotype analyses were done for DA1 and 5-HT2A receptor genes in PD-pure subgroup (cases + controls, n=188). Tables 4–6 present all statistically relevant data on the detected haplotype-phenotype associations with PD-all and PD-pure phenotypes.

Four haplotypes were found for CCK gene in PD-all group with a major haplotype and a relatively common haplotype 2 (Table 4). The reference haplotype, which combines the wild-type alleles at each locus, was slightly more frequent in controls, whereas haplotype 2 was significantly more frequent in patients. Haplotype 2 (TG) was associated with a higher risk of PD (OR=1.77; p=0.04) than the reference haplotype. This association reflects a higher frequency of both SNP –45T and 1270G alleles in cases compared to controls. The global haplotypic effect of CCK gene did not reach the level of statistical significance (χ^2=5.23, df=2; p=0.073).

Six haplotypes were found for DA1 receptor gene in the PD-pure group (Table 5) with two major haplotypes: the reference haplotype 1, which combines the most frequent alleles at each polymorphic site, and a common haplotype 2 combining the wild-type alleles at each locus. Together with haplotype 3 these haplotypes constituted more than 90% of all alleles. The reference haplotype was found in a significantly higher proportion of affected individuals, whereas haplotype 3 was significantly more frequent in controls. Haplotype 5 was not inferred. Haplotype 3 (CAA) was associated with a lower risk of PD-pure phenotype (OR=0.25; p=0.03) as compared to the reference haplotype. This association mainly reflects a higher frequency of –94A allele in controls indicating an individual SNP –94G-A effect of a marginal significance. The test of a global DA1 haplotypic effect demonstrated association with pure PD phenotype (χ^2=9.02, df=3; P=0.029). The haplotype count and distribution in the PD-all group were similar to the PD-pure group, except for haplotypes 1 and 3, where differences in haplotype frequencies between cases and controls were much less remarkable (40.6% vs. 44.4% and 14.5% vs. 9.5% respectively). Therefore, the haplotype-phenotype effect for haplotype 3 (CAA) was very weak (OR=0.58; P=0.089). Thus, in the case of DA1 receptor gene, the haplotype-based strategy detected an involvement of –94G-A in the genetic predisposition to PD.

Four two-marker haplotypes were found for 5-HT2A receptor gene in the PD-pure group (Table 6) with a major reference haplotype, which combined the most frequent alleles, and a common haplotype 2 combining the wild-type alleles at each locus. These haplotypes constituted more than 85% of all alleles. The reference haplotype (GC) was found in a higher proportion of affected individuals, whereas haplotype 2 (AT) was significantly more frequent in controls. Haplotype 4 was not inferred. Haplotype 2 was associated with a lower risk of PD (OR=0.49; p=0.04) than the reference haplotype. This association reflected in part a higher frequency of 102T allele and 102TT genotype in controls. The individual risk effect for SNP 102T-C in a comparison of haplotype 2 (AT) *vs* 3 (AC) was not statistically significant (OR=1.8; p=0.23). Despite the fact that for SNP –1438A-G the individual haplotype effect remained not significant, an interaction between –1438A-G and 102T-C allelic variants seemed to determine to a large extent the actual gene dose effect for 5-HT2A receptor. These markers have been consistently reported to be in linkage

disequilibrium (D'=0.84 in our study population). The test of the global 5-HT2A haplotypic association with PD was not significant (χ^2=5.54, df=3; p=0.14).

5.6.3. Discussion

The results of this study suggest that several genes of various neurotransmitter systems, each of a minor individual contribution, might contribute to the susceptibility to PD. The data also illustrates that the genetic variability in candidate genes may have a distinctive influence on pure and comorbid phenotypes of PD.

The previous studies had excluded a major role of several 5-HT-related gene polymorphisms in PD. The negative results were described for 5-HT2C receptor Cys23Ser and 5-HT1B receptor 861G-C variants (Fehr et al., 2000 a, b), Fnu4H1 polymorphism of the MAO-A (Tadic et al., 2003), 218A-C and 1095T-C of TPH (Fehr et al., 2001; Han et al., 1999), and 5-HTTLPR (Deckert et al., 1997). However, one study found a positive association between MAO-A gene promoter polymorphism and PD (Deckert et al., 1999). Recently, Inada et al. (2003) detected a significant association of 5-HT2A receptor 102T-C polymorphism with pure PD although this association was more significant in PD patients with agoraphobia. Our study also showed an association between the 5-HT2A receptor silent 102T-C polymorphism and pure PD but not comorbid PD phenotype. Thus, the data support the role of 5-HT2A receptor 102T-C polymorphism and haplotype variants formed by linked SNPs –1438A-G/102T-C in the genetic predisposition to PD. The studies of this SNP in other psychiatric disorders have so far shown lack of association with mood disorders, suicidality or treatment response to antidepressants (Geijer et al., 2000; Massat et al., 2000; Cusin et al., 2002; Oswald et al., 2003). However, there are some positive findings on its association with schizophrenia and response to clozapine treatment (Williams et al., 1996; Arranz et al., 1997).

The results of previous studies argued against the major role of 5-HT2C receptor Cys23Ser polymorphism in PD and indicated its possible role in major depression and bipolar disorder (Fehr et al., 2000a; Lerer et al., 2001; Inada et al., 2003). In the present study this polymorphism was not associated with PD in the subgroup comorbid with unipolar major depression. However, an association was detected in the whole PD group that included all patients with or without affective comorbidity. The recently identified 5-HT1A receptor –1018C-G polymorphism was not associated with major depression in one previous study (Arias et al., 2002) but was recently linked to a predisposition to depression and suicide (Lemonde et al., 2003). Our study demonstrated an association of this SNP with PD in the whole group as well as in PD comorbid with affective disorders, but not in pure PD. Interestingly, in a recent case-control study, Rothe et al. (2004) have found a significant association of this

polymorphism with PD comorbid with agoraphobia, but not in the total sample of PD patients.

Altogether the data of this study suggest that certain gene variants of 5-HTergic and DA-ergic systems may have a more specific role in genetic predisposition to PD. The polymorphism in CCK-related genes appears to have a greater influence in PD with affective comorbidity. In particular, the distinctive association between 5-HT2A receptor 102T-C polymorphism and pure PD may indicate specific influence of this genetic variant on predisposition to PD, whereas 5-HT2C Cys23Ser and 5-HT1A –1018C-G receptor polymorphisms may have non-specific links with comorbid phenotypes of PD. However, the small sample size of the PD-pure subgroup may have impaired the power of this study to detect more subtle effects of other candidate genes. It should be also emphasized that this study carries a risk of false positive findings due to the large number of comparisons performed in the analysis. Possible population stratification in the present study also cannot be excluded. Further independent studies in different populations including family-based association studies in substantially larger number of individuals with different subtypes of PD will be necessary to verify and extend these results. Methodologically, our study demonstrated that high-throughput DNA microarray-based genotyping method, such as APEX, may be a valuable tool in detecting the associations with markers from multiple candidate genes of various neurotransmitter systems as well as in the identification of the role of newly described polymorphisms. Future studies using this technology could generate novel hypotheses and expand our knowledge of the role of genetic regulation of neurotransmitter systems in the expression and complexity of psychiatric disorders, including PD.

6. GENERAL DISCUSSION

The presented studies were conducted in order to investigate the 5-HT role in susceptibility and pathogenesis of PD. The results of the studies support the important role of 5-HT system in neurobiology of panic attacks and PD. We found that an increase in 5-HT availability by acute administration of 5-HTP significantly reduced the rate of CCK-4-induced panic attacks and cognitive symptoms in females and lowered the somatic panic symptoms in male healthy subjects. Thus in line with previous findings (Klaassen et al., 1998; Schruers et al., 2000, 2002) the results of this study provide further support to the hypothesis of a specific inhibitory influence of 5-HT on panic responses (Deakin and Graeff, 1991). Although it is quite clear that acute pretreatment with 5-HTP increases the net amount of released 5-HT and its synaptic availability (Dreshfield-Ahmad et al., 2000; Fickbohm and Katz, 2000), the exact antipanic mechanism of 5-HTP needs further study. An increased synaptic 5-HT neurotransmission plays a central role in the antipanic effects of SSRIs (Nutt et al., 1999), and it was proposed that antipanic action of antidepressants could be due to facilitation of the DRN-periventricular 5-HT pathway, resulting in the overstimulation of 5-HT2A or 5-HT1A receptors in the PAG (Deakin and Graeff, 1991). Moreover, MRN innervation of the hippocampal – amygdaloid complex, the primary limbic recipient of viscerosensory afferents, exerts inhibitory effects via 5-HT1A receptors (Blier et al., 1987; Schreiber and DeVry, 1993). Thus, the antipanic effects of 5-HTP may be mediated via 5-HT2A or 5-HT1A receptors. There is also evidence that an increase in plasma cortisol and prolactin following 5-HTP administration in man are modulated by at least three different postsynaptic receptors, the 5-HT1A, 5-HT2A, 5-HT2C and 5-HT3 receptors (Meltzer and Maes, 1994; Meltzer et al., 1997). Moreover, pretreatment with pindolol, a 5-HT1A partial antagonist significantly inhibited the prolactin but not the cortisol response to 5-HTP (Meltzer and Maes, 1994). However, ritanserin, a 5-HT2A/5-HT2C antagonist, did not block the prolactin response to tryptophan (Deakin, 1996). Furthermore, subchronic treatment with SSRI, fluoxetine, but not tricyclic antidepressants, significantly enhanced 5-HTP-induced cortisol and prolactin responses in patients with major depression or obsessive-compulsive disorder (Meltzer et al., 1997). Nevertheless, it is not clear whether neuroendocrine and antipanic effects of 5-HTP are mediated via the same 5-HTergic receptors. Besides 5-HT, other neurotransmitters and CNS chemicals, such as melatonin, DA, NOR, and beta-endorphin have also been shown to increase after oral administration of 5-HTP (van Praag et al., 1987; den Boer and Westenberg, 1990a; Guilleminault et al., 1973). Therefore, future experimental studies with selective 5-HTergic antagonists could be useful to clarify the antipanic action of 5-HTP.

The antipanic effect of 5-HTP in our and another recent study (Schruers et al., 2002) occurred 2 h after a single administration, which seems at odds with findings of clinical studies with 5-HT transmission-enhancing drugs like SSRIs, showing an antipanic effect only after several weeks of treatment. However, there have been no data regarding effect of single aministration of SSRIs on the experimentally induced panic. Also, there is a fundamental difference between 5-HTP and SSRIs in their mechanisms for increasing the 5-HT availability. The 5-HTP raises the synaptic concentration of 5-HT directly by increasing the 5-HT synthesis rate that may be decreased in PD. In contrast, the SSRIs increase 5-HT amount due to inhibition of 5-HT reuptake resulting in desensitization of different feedback systems, although their possibility to induce new synthesis and activity of TPH should be also taken into account (Briley and Moret, 1993). Thus, enhancement of 5-HT under the conditions of a low synaptic availability of 5-HT due to a rise in 5-HT synthesis is probably a quicker way to achieve the antipanic effect than an accumulative increase in synaptic 5-HT by its reuptake inhibition. Moreover, earlier studies demonstrated that treatment with 5-HTP had beneficial effects on the panic attacks in patients with anxiety disorders (Kahn and Westenberg, 1985; Kahn et al., 1987). Additionally, the therapeutic administration of 5-HTP has been demonstrated as beneficial and fast-acting in the treatment of depression (Birdsall, 1998). Taken together, 5-HTP may have a therapeutic effect in PD that warrants further study.

A central question, which needs more explanation is whether there is a deficit in 5-HT synthesis or/and in 5-HT neurotransmission in patients with PD. The results of our and other brain imaging studies (Nutt et al., 2003; Neumeister et al., 2004) show that the reduced density of 5-HTT and 5-HT1A receptors in the brain of patients with PD may reflect a deficit in 5-HT neurotransmission and/or a compensatory process in the 5-HT system attempting to increase the availability of synaptic 5-HT. Furthermore, the associations between PD and genetic variants, which probably lead to the decreased synaptic 5-HT availability, provide additional support for the possible existence of deficient 5-HT neurotransmission in PD. However, it is not clear as yet whether reduction in 5-HTT and 5-HT1A receptors might have any region-specific influence on the 5-HT neurotransmission in PD. Moreover, the persisting reduction of midbrain 5-HT1A receptors and thalamic 5-HTT in remitted PD patients seems intriguing in light of the 5-HT deficit hypothesis of PD. Thus further brain imaging studies to measure the 5-HT synthesis rate are needed for further characterization of 5-HT availability and neurotransmission in PD patients in different stages of disease. Interestingly, previous PET studies demonstrated a suppressed 5-HT synthesis rate indexed by a significantly lower brain uptake of radiolabelled 5-HTP in patients with social anxiety disorder as well as in patients with major depression as compared to healthy subjects (Agren et al., 1991; Marteinsdottir et al., 2001). Besides the putative 5-HT synthesis deficit, various other alterations, such as abnormal conversion of L-tryptophan, impaired storage, release or increased destruction of 5-HT, and dysregulation of

some receptors (for example, alpha 2-adrenoceptor) may lead to lowering of 5-HT availability or transmission in PD.

The reduced binding of 5-HTT and 5-HT1A receptors in PD patients in remission raises the possibility that these alterations are characteristic of the disease or perhaps serve as markers for a primary aetiologic defect in the development and/or functioning of the 5-HT system. Animal studies support this suggestion showing that mice lacking 5-HT1A receptors display enhanced anxiety (Ramboz et al., 1998; Parks et al., 1998). However, no differences in anxiety-related behaviours were seen in the open field and the elevated plus maze in 5-HTT knockout mice (Lira et al., 2003). Interestingly, 5-HT1A receptor knockout mice did not differ from wild-type mice in the density of 5-HTT or post-synaptic 5-HT2A receptors, and an index of 5-HT neuronal activity (He et al., 2001), although the 5-HT1A receptor levels were found significantly decreased in the DRN, increased in the hippocampus, and unchanged in other forebrain areas of 5-HTT knockout mice (Fabre et al., 2000). Nevertheless, the knockout models in animals probably reflect anxiety rather than panic and therefore may have different significance than adaptive changes in the 5-HT system seen in patients with PD. Another consideration is that reduced 5-HTT and 5-HT1A receptor expression in PD are associated with a genetic risk factor for PD. Notably, a 5-HT1A receptor -1018C-G polymorphism and 5-HTT promoter region polymorphism that respectively regulate 5-HT1A receptor and 5-HTT transcription, both have been associated with PD in our and other recent genetic studies. However, the association between PD phenotype and 5-HTT polymorphism leading to higher protein expression seems to contradict the hypothesis of a compensatory reduction of 5-HTT expression in PD patients. Probably, this inconsistency may be explained by a variable role of high-expressive 5-HTTLPR long allele in the course of PD. The long allele genotype, in particular, might be associated with the predisposition to PD, but due to a higher transcriptional activity it may allow greater compensatory propensity in the pathogenesis of PD. Pertinently, the long allele variant of 5-HTTLPR has been associated with a better response to treatment with SSRIs in females with PD (Catalano, 2001). In accordance with this suggestion some studies found associations between 5-HTTLPR long allele genotype and other disorders putatively involving the deficiency of 5-HT neurotransmission, such as alcoholism (Parsian and Cloninger, 2001). Furthermore, a recent SPECT study demonstrated reduced midbrain 5-HTT availability in patients with alcoholism, whereas the LL-homozygous alcoholic subjects showed a significantly lower midbrain 5-HTT availability compared to LL-homozygous controls (Heinz et al., 2000). These data indicate that the importance to study the relationship between functional genetic variants of 5-HT system and characteristics of 5-HTergic genes expression is high on the agenda. It would be also of interest to study the influence of treatment on the 5-HT related gene expression in patients with PD.

The results of our genetic association studies suggest that genes of 5-HT system might contribute to the susceptibility to PD. However, the 5-HTergic genetic impact seems to be differently related to PD phenotypes and may be gender-related. This indicates importance of better distinction between PD phenotypes and the necessity to take into account gender differences in further investigations of genetic and biological backgrounds in PD. Methodologically, we have shown that high-throughput DNA microarray-based genotyping method, such as APEX, is a valuable tool for future genetic association studies. Use of this method might contribute to a fast expansion of our knowledge of the role of genetic regulation of 5-HT and other neurotransmitter systems in the expression and complexity of PD. In particular, it seems promising to use the APEX technology in the pharmacogenetic studies aiming to clarify the influences of the 5-HT-related gene polymorphisms on the outcomes of PD treatment.

It is important to consider that 5-HT system affects many other neurotransmitters in key brain structures involved in the processing and expression of panic and anxiety (Coplan and Lydiard, 1998; Kent et al., 1998). Therefore, future studies should focus on the relationship between the 5-HT and other neurotransmitter systems and their interactive impact on panic neurocircuitry. In addition, the further delineation of the region-specific role of 5-HTergic projections from the MRN and DRN is needed for more specific characterization of 5-HT impact on PD complexity and subtypes. It also means that involvement of 5-HT in cognitive background of PD warrants further investigations. Finally, it needs to be emphasized that PD is probably a biologically heterogeneous condition with important psychological components. It would be an over-simplification to consider 5-HT dysfunction as the primary aetiological factor in PD. However, a better understanding of 5-HT function will lead toward a better understanding of the origins of pathological anxiety and improved clinical management of PD.

7. CONCLUSIONS

1. Acute pretreatment with 5-HT precursor 5-HTP significantly reduced the rate of CCK-4-induced panic attacks and cognitive symptoms in females and lowered the somatic panic symptoms in male healthy subjects.
2. The CCK-4 panic rate was associated with functional polymorphisms of MAO-A and 5-HTT genes in female but not male healthy subjects. These results provide further support to the hypothesis of the specific inhibitory influence of 5-HT on panic attacks and indicate the importance of further studies into gender differences of role of 5-HT in PD neurobiology.
3. The PD patients showed reduction in the density of brain 5-HTT related to the clinical status. It may reflect a deficit of neuronal 5-HT or a compensatory process in the 5-HT system attempting to increase the availability of synaptic 5-HT. However, GAD patients demonstrated unaffected binding of brain 5-HTT, suggesting a different functional role of 5-HTT in GAD than in PD.
4. As compared to healthy subjects, patients with PD revealed over-representation of the high-expressed variant of 5-HTTLPR although the non-functional 5-HTergic polymorphisms such as 5-HTT VNTR, TPH 218A/C and 5-HT1B receptor 861G/C, were not associated with PD. Additionally, a significantly higher frequency of the transcriptionally more active MAO-A promoter polymorphism was demonstrated in agoraphobic PD females. These findings suggest that genetic variants conceivably related to lower 5-HT neurotransmission may be involved in the development of PD.
5. The broad screening of candidate genes indicated that certain gene variants of 5-HTergic system, such as 5-HT2A receptor 102T-C polymorphism, may have a more specific role in the genetic predisposition to PD, whereas others, such as 5-HT2C Cys23Ser and 5-HT1A –1018C-G receptor polymorphisms appeared to have a stronger influence on PD comorbid with mood disorders. The results of the genetic studies underline the importance of differentiating distinctive comorbidity patterns in order to better define the genetically explained phenotypes of PD.

8. REFERENCES

1. Acton P, Kushner S, Kung M, Mozley P, Plossl K, Kung H (1999) Simplified reference region model for the kinetic analysis of [99mTc]TRODAT-1 binding to dopamine transporters in nonhuman primates using single-photon emission tomography. Eur J Nucl Med 26: 518–526.
2. Agren H, Reibring L, Hartvig P, Tedroff J, Bjurling P, Hornfeldt K, Andersson Y, Lundqvist H, Langstrom B (1991) Low brain uptake of L-[11C]5-hydroxy-tryptophan in major depression: a positron emission tomography study on patients and healthy volunteers. Acta Psychiatr Scand 83: 449–455.
3. Amaral D, Insausti R (1992) Retrograde transport of D-[3H]-aspartate injected into the monkey amygdaloid complex. Exp Brain Res 88: 375–388.
4. Amaral D, Price J, Pitkanen A, Carmichael S (1992) Anatomical organization of the primate amygdaloid complex. In: Aggleton JP (ed). The Amygdala: Neuro-biological Aspects of Emotion, Memory and Mental Dysfunction. NY Wiley-Liss: 1–66.
5. American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Washington, DC: American Psychiatric Association.
6. Amering M, Bankier B, Berger P, Griengl H, Windhaber J, Katschnig H (1999) Panic disorder and cigarette smoking behavior. Compr Psychiatry 40: 35–38.
7. Apostolopoulos M, Judd F, Burrows G, Norman T (1993) Prolactin response to dl-fenfluramine in panic disorder. Psychoneuroendocrinology 18: 337–342.
8. Arbelle S, Benjamin J, Golin M, Kremer I, Belmaker R, Ebstein R (2003) Relation of shyness in grade school children to the genotype for the long form of the serotonin transporter promoter region polymorphism. Am J Psychiatry 160: 671–676.
9. Arias B, Arranz M, Gasto C, Catalan R, Pintor L, Gutierrez B, Kerwin R, Fananas L (2002) Analysis of structural polymorphisms and C-1018G promoter variant of the 5-HT(1A) receptor gene as putative risk factors in major depression. Mol Psychiatry 7: 930–932.
10. Arranz M, Munro J, Sham P, Zhao J, Kirov G, Sodhi M, Spurlock G, Owen M, Collier D, Kerwin R (1997) Polymorphisms in the 5-HT2A receptor gene and promoter region associated with clozapine response. Schizophr Res 24: 90.
11. Azmitia EC, Whitaker PM (1995) Anatomy, cell biology, and plasticity of the serotonergic system: Neuropsychopharmacological implications for the actions of psychotropic drugs. In: Bloom FE, Kupfer DJ (eds). Psychopharmacology: The Fourth Generation of Progress. NY Raven Press: 443– 490.
12. Bandler R, Carrive P (1988) Integrated defence reaction elicited by excitatory amino acid microinjection in the midbrain periaqueductal grey region of the unrestrained cat. Brain Res 439: 95–106.
13. Barlow DH (1988) Anxiety and its Disorders: The Nature and Treatment of Anxiety and Panic. NY: Guilford Press.
14. Battersby S, Ogilvie A, Smith C, Blackwood D, Muir W, Quinn J, Fink G, Goodwin G, Harmar A (1996) Structure of a variable number tandem repeat of the serotonin transporter gene and association with affective disorder. Psychiatr Genet 6: 177–181.

15. Baumgarten HG, Göthert M (2000) Serotoninergic neurons and 5-HT receptors in the CNS. Second printing. Springer-Verlag Berlin Heidelberg.
16. Bell C, Nutt D (1998) Serotonin and panic. Br J Psychiatry 172: 465–471.
17. Bell C, Forshall S, Adrover M, Nash J, Hood S, Argyropoulos S, Rich A, Nutt D (2002) Does 5-HT restrain panic? A tryptophan depletion study in panic disorder patients recovered on paroxetine. J Psychopharmacol 16: 5–14.
18. Bellivier F, Henry C, Szoke A, Schurhoff F, Nosten-Bertrand M, Feingold J, Launay J, Leboyer M, Laplanche J (1998) Serotonin transporter gene polymorphisms in patients with unipolar or bipolar depression. Neurosci Lett 255: 143–146.
19. Bengel D, Greenberg BD, Cora-Locatelli G, Altemus M, Heils A, Li Q, Murphy D (1999) Association of the serotonin transporter promoter regulatory region polymorphism and obsessive-compulsive disorder. Mol Psychiatry 4: 463–466.
20. Bergström K, Halldin C, Hall H, Lundkvist C, Ginovart N, Swahn C, Farde L (1997) In vitro and in vivo characterization of nor-ß-CIT: a new radioligand for visualization of the serotonin transporter in brain. Eur J Nucl Med 24: 596–601.
21. 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.
22. Birdsall T (1998) 5-Hydroxytryptophan: a clinically-effective serotonin precursor. Altern Med Rev 3: 271–280.
23. Bisaga A, Katz J, Antonini A, Wright C, Margouleff C, Gorman J, Eidelberg D (1998) Cerebral glucose metabolism in women with panic disorder. Am J Psychiatry 155: 1178–1183.
24. Blier P, de Montigny C, Chaput Y (1987) Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. J Clini Psychopharmacol 7: 24–35.
25. Blier P, Seletti B, Gilbert F, Young S, Benkelfat C (2002) Serotonin 1A receptor activation and hypothermia in humans: lack of evidence for a presynaptic mediation. Neuropsychopharmacology 27: 301–308.
26. Boshuisen M, Ter Horst G, Paans A, Reinders A, den Boer J (2002) rCBF differences between panic disorder patients and control subjects during anticipatory anxiety and rest. Biol Psychiatry 52: 126–135.
27. Boulenger J, Uhde T (1982) Caffeine consumption and anxiety: preliminary results of a survey comparing patients with anxiety disorders and normal controls. Psychopharmacol Bull 18: 53–57.
28. Bourin M, Baker G, Bradwejn J (1998) Neurobiology of panic disorder. J Psychosom Res 44: 163–180.
29. Bradwejn J, Koszycki D (1994a) The cholecystokinin hypothesis of anxiety and panic disorder. Ann N Y Acad Sci 713: 273–282.
30. Bradwejn J, Koszycki D (1994b) Imipramine antagonism of the panicogenic effects of cholecystokinin tetrapeptide in panic disorder patients. Am J Psychiatry 151: 261–263.
31. Bradwejn J, Koszycki D, Shriqui C (1991) Enhanced sensitivity to cholecystokinin tetrapeptide in panic disorder. Clinical and behavioral findings. Arch Gen Psychiatry 48: 603–610.

32. Bremner J, Innis R, White T, Fujita M, Silbersweig D, Goddard A, Staib L, Stern E, Cappiello A, Woods S, Baldwin R, Charney D (2000) SPECT [I-123]iomazenil measurement of the benzodiazepine receptor in panic disorder. Biol Psychiatry 47: 96–106.
33. Bressa G, Marini S, Gregori S (1987) Serotonin S2 receptors blockage and generalized anxiety disorders. A double-blind study on ritanserin and lorazepam. Int J Clin Pharmacol Res 7: 111–119.
34. Briley M, Moret C (1993) Neurobiological mechanisms involved in antidepressant therapies. Clin Neuropharmacol 16: 387–400.
35. Broocks A, Bandelow B, George A, Jestrabeck C, Opitz M, Bartmann U, Gleiter C, Meineke I, Roed I, Ruther E, Hajak G (2000) Increased psychological responses and divergent neuroendocrine responses to m-CPP and ipsapirone in patients with panic disorder. Int Clin Psychopharmacol 15: 153–161.
36. Broocks A, Meyer T, Opitz M, Bartmann U, Hillmer-Vogel U, George A, Pekrun G, Wedekind D, Ruther E, Bandelow E (2003) 5-HT1A responsivity in patients with panic disorder before and after treatment with aerobic exercise, clomipramine or placebo. Eur Neuropsychopharmacol 13: 153–164.
37. Candilis P, McLean R, Otto M, Manfro G, Worthington J, Penava S, Marzol P, Pollack M (1999) Quality of life in patients with panic disorder. J Nerv Ment Dis 187: 429–434.
38. Carli M, Afkhami-Dastjerdian S, Reader T (1996) [3H]8-OH-DPAT binding and serotonin content in rat cerebral cortex after acute fluoxetine, desipramine, or pargyline. J Psychiatry Neurosci 21: 114–122.
39. Catalano M (2001) Functionally gene-linked polymorphic regions and genetically controlled neurotransmitters metabolism. Eur Neuropsychopharmacol 11: 431–439.
40. Chapman W, Schroeder, H, Guyer G et al (1954) Physiological evidence concerning the importance of the amygdaloid nuclear region in the integration of circulating function and emotion in man. Science 129: 949 –950.
41. Charney D, Drevets W (2002) Neurobiological basis of anxiety disorders. In: Neuropsychopharmacology: the fifth generation of progress (Davis K, Charney D, Coyle J, Nemeroff C, eds), 901–930. Philadelphia: Lippincott Williams and Wilkins.
42. Charney D, Heninger G (1986) Serotonin function in panic disorders. The effect of intravenous tryptophan in healthy subjects and patients with panic disorder before and during alprazolam treatment. Arch Gen Psychiatry 43: 1059–1065.
43. Charney D, Heninger G, Jatlow P (1985) Increased anxiogenic effects of caffeine in panic disorders. Arch Gen Psychiatry 42: 233–243.
44. Charney D, Woods S, Goodman W, Heninger G (1987) Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. Psychopharmacologia 92: 14–24.
45. Charney D, Woods S, Krystal J, Nagy L, Heninger G (1992) Noradrenergic neuronal dysregulation in panic disorder: the effects of intravenous yohimbine and clonidine in panic disorder patients. Acta Psychiatr Scand 86: 273–282.
46. Chen Y, Dilsaver S (1995) Comorbidity of panic disorder in bipolar illness: evidence from the Epidemiologic Catchment Area Survey. Am J Psychiatry 152: 280–282.

47. Clark DM (1988) A cognitive model of panic. In Rachman S, Maser J (eds). Panic: Psychological Perspectivies. Hillsdale, NJ Erlbaum.
48. Collier D, Stober G, Li T, Heils A, Catalano M, Di Bella D, Arranz M, Murray R, Vallada H, Bengel D, Muller C, Roberts G, Smeraldi E, Kirov G, Sham P, Lesch K (1996) A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. Mol Psychiatry 1: 453–460.
49. Coplan J, Gorman J, Klein D (1992) Serotonin related functions in panic-anxiety: a critical overview. Neuropsychopharmacology 6: 189–200.
50. Coplan J, Lydiard R (1998) Brain circuits in panic disorder. Biol Psychiatry 44: 1264–1276.
51. Cutler N, Hesselink J, Sramek J (1994) A phase II multicenter dose-finding, efficacy and safety trial of ipsapirone in outpatients with generalized anxiety disorder. Prog Neuropsychopharmacol Biol Psychiatry 18: 447–463.
52. Cusin C, Serretti A, Zanardi R, Lattuada E, Rossini D, Lilli R, Lorenzi C, Smeraldi E (2002) Influence of monoamine oxidase A and serotonin receptor 2A polymorphisms in SSRI antidepressant activity. Int J Neuropsychopharmacol 5: 27–35.
53. Davis M (1992) The role of the amygdala in fear and anxiety. Annu Rev Neurosci 15: 353–375.
54. De Cristofaro M, Sessarego A, Pupi A, Biondi F, Faravelli C (1993) Brain perfusion abnormalities in drug-naive, lactate-sensitive panic patients: a SPECT study. Biol Psychiatry 33: 505–512.
55. Deakin JFW (1996) 5-HT, antidepressant drugs and the psychosocial origins of depression. J Psychopharmacol 10: 31–38.
56. Deakin JFW, Connell, Anderson I, Miller H (1996) Experimental models of anxiety. Eur Psychiatry 11: 209.
57. Deakin JFW, Graeff F (1991) 5-HT and mechanisms of defence. J Psychopharmacol 5: 305–315.
58. Deckert J, Catalano M, Heils A, Di Bella D, Friess F, Politi E, Franke P, Nöthen M, Maier W, Bellodi L, Lesch KP (1997) Functional promoter polymorphism of the human serotonin transporter: lack of association with panic disorder. Psychiatr Genet 7: 45–47.
59. Deckert J, Catalano M, SyagailoY, Bosi M, Okladnova O, Di Bella D, Nöthen M, Maffei P, Franke P, Fritze J, Maier W, Propping P, Beckmann H, Bellodi L, Lesch KP (1999) Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum Mol Genet 8: 621–624.
60. Deckert J, Meyer J, Catalano M, Bosi M, Sand P, Di Bella D, Ortega G, Stober G, Franke P, Nothen M, Fritze J, Maier W, Beckmann H, Propping P, Bellodi L, Lesch KP (2000). Novel 5´-regulatory region polymorphisms of the 5-HT2C receptor gene: association study with panic disorder. Int J Neuropsychopharmacol 4: 321–325.
61. Deckert J, Nothen M, Franke P, Delmo C, Fritze J, Knapp M, Maier W, Beckmann H, Propping P (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.

62. den Boer JA, Westenberg HG (1990a) Behavioral, neuroendocrine, and biochemical effects of 5-hydroxytryptophan administration in panic disorder. Psychiatry Res 31: 267–278.
63. den Boer JA, Westenberg HG (1990b) Serotonin function in panic disorder: a double blind placebo controlled study with fluvoxamine and ritanserin. Psychopharmacologia 102: 85–94.
64. de Olmos J (1990) Amygdaloid nuclear gray complex. In: Paxinos G (ed). The Human Nervous System. San Diego, CA Academic Press: 583–710.
65. Depot M, Merani S, Bradwejn J, Mukherjee J, Caille J, Gutkowska J, Caille G (1998) Effect of oral ondansetron on total cholecystokinin plasma levels following CCK-4 panic challenge procedure in healthy men. J Psychiatry Neurosci 23: 298–304.
66. Dickenson J, Hill S (1998) Human 5-HT1B receptor stimulated inositol phospholipid hydrolysis in CHO cells: synergy with Gq-coupled receptors. Eur J Pharmacol 348: 279–285.
67. Dreshfield-Ahmad L, Thompson D, Schaus J, Wong D (2000) Enhancement in extracellular serotonin levels by 5-hydroxytryptophan loading after administration of WAY 100635 and fluoxetine. Life Sci 66: 2035–2041.
68. Drevets W, Frank E, Price J, Kupfer D, Greer P, Mathis C (2000) Serotonin type-1A receptor imaging in depression. Nucl Med Biol 27: 499–507.
69. Eaton W, Kessler R, Wittchen H, Magee W (1994) Panic and panic disorder in the United States. Am J Psychiatry 151: 413–420.
70. Eren I, Tukel R, Polat A, Karaman R, Unal S (2003) Evaluation of regional cerebral blood flow changes in panic disorder with Tc99m-HMPAO SPECT. Psychiatry Res 123: 135–143.
71. Eriksson E (1993) Brain neurotransmission in panic disorder. Acta Psychiatr Scand 335 Suppl: 31–37.
72. Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP, Murphy D, Lanfumey L, Hamon M, Martres M (2000) Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. Eur J Neurosci 12: 2299–2310.
73. Faludi G, Tekes K, Tothfalusi L (1994) Comparative study of platelet 3H-paroxetine and 3H-imipramine binding in panic disorder patients and healthy controls. J Psychiatry Neurosci 19: 109–113.
74. Faravelli C (1985) Life events preceding the onset of panic disorder. J Affect Disord 9: 103–105.
75. Faravelli C, Paterniti S, Scarpato A (1995) 5-year prospective, naturalistic follow-up study of panic disorder. Compr Psychiatry 36: 271–277.
76. Fehr C, Grintschuk N, Szegedi A, Anghelescu I, Klawe C, Singer P, Hiemke C, Dahmen N (2000a) The HTR1B 861G>C receptor polymorphism among patients suffering from alcoholism, major depression, anxiety disorders and narcolepsy. Psychiatry Res 97: 1–10.
77. Fehr C, Schleicher A, Szegedi A, Anghelescu I, Klawe C, Hiemke C, Dahmen N (2001) Serotonergic polymorphisms in patients suffering from alcoholism, anxiety disorders and narcolepsy. Prog Neuropsychopharmacol Biol Psychiatry 25: 965–982.
78. Fehr C, Szegedi A, Anghelescu I, Klawe C, Hiemke C, Dahmen N (2000b) Sex differences in allelic frequencies of the 5-HT2C Cys23Ser polymorphism in

psychiatric patients and healthy volunteers: findings from an association study. Psychiatr Genet 10: 59–65.
79. Fickbohm D, Katz P (2000) Paradoxical actions of the serotonin precursor 5-hydroxytryptophan on the activity of identified serotonergic neurons in a simple motor circuit. J Neurosci 20: 1622–1634.
80. Fischer H, Andersson J, Furmark T, Fredrikson M (1998) Brain correlates of an unexpected panic attack: a human positron emission tomographic study. Neurosci Lett 251: 137–140.
81. Fontaine R, Breton G, Dery R, Fontaine S, Elie R (1990) Temporal lobe abnormalities in panic disorder: an MRI study. Biol Psychiatry 27: 304–310.
82. Fowler J, MacGregor R, Wolf A, Arnett C, Dewey S, Schlyer D, Christman D, Logan J, Smith M, Sachs H et al (1987) Mapping human brain monoamine oxidase A and B with 11C-labeled suicide inactivators and PET. Science 235: 481–485.
83. Frazer A, Hensler J (1990) 5-HT1A receptors and 5-HT1A-mediated responses: effect of treatments that modify serotonergic neurotransmission. Ann N Y Acad Sci 600: 460–474.
84. Frazer G, Lapierre Y (1987) The effect of buspirone on panic disorder: a case report. J Clin Psychopharmacol 7: 118–119.
85. Geijer T, Frisch A, Persson M, Wasserman D, Rockah R, Michaelovsky E, Apter A, Jonsson E, Nothen M, Weizman A (2000). Search for association between suicide attempt and serotonergic polymorphisms. Psychiatr Genet 10: 19–26.
86. George D, Nutt D, Rawlings R, Phillips M, Eckardt M, Potter W, Linnoila M (1995) Behavioral and Endocrine Responses to Clomipramine in Panic Disorder Patients with or without Alcoholism. Biol Psychiatry 37: 112–119.
87. Germine M, Goddard A, Woods S, Charney D, Heninger G (1992) Anger and anxiety responses to m-chlorophenylpiperazine in generalized anxiety disorder. Biol Psychiatry 32: 457–461.
88. Germine M, Goddard A, Sholomskas D, Woods S, Charney D, Heninger G (1994) Response to meta-chlorophenylpiperazine in panic disorder patients and healthy subjects: Influence of reduction in intravenous dosage. Psychiatry Res 54: 115–133.
89. Glatt C, Freimer N (2002) Association analysis of candidate genes for neuropsychiatric disease: the perpetual campaign. Trends Genet 18:307–312.
90. Goisman R, Warshaw M, Peterson L, Rogers M, Cuneo P, Hunt M, Tomlin-Albanese J, Kazim A, Gollan J, Epstein-Kaye T et al (1994) Panic, agoraphobia, and panic disorder with agoraphobia. Data from a multicenter anxiety disorders study. J Nerv Ment Dis 182:72–79.
91. Goldstein R, Wickramarante P, Horwath E, Weissman M (1997) Familial aggregation and phenomenology of "early"-onset (at or before age 20 years) panic disorder. Arch Gen Psychiatry 54: 271–278.
92. Gorman J, Askanazi J, Liebowitz M, Fyer A, Stein J, Kinney J, Klein D (1984) Response to hyperventilation in a group of patients with panic disorder. Am J Psychiatry 141: 857–861.
93. Gorman J, Kent J, Sullivan G, Coplan J (2000) Neuroanatomical hypothesis of panic disorder, revised. Am J Psychiatry 157: 493–505.
94. Gorman J, Liebowitz M, Fyer A, Stein J (1989) A neuroanatomical hypothesis for panic disorder. Am J Psychiatry 146: 148–161.

95. Graeff FG (1990) Brain defence systems and anxiety. In: Roth M, Burrows GD, Noyes R (eds). Handbook of Anxiety, vol 3. Amsterdam Elsevier Science Publishers: 307–357.
96. Graeff FG (1993) Role of 5-HT in defensive behavior and anxiety. Rev Neurosci 4: 181–211.
97. Graeff FG (1997) Serotonergic systems. Psychiatr Clin North Am 20: 723–739.
98. Graeff FG, Guimaraes F, De Andrade T, Deakin JFW (1996) Role of 5-HT in Stress, Anxiety, and Depression. Pharmacol Biochem Behav 54: 129–141.
99. Gray JA (1988) The neuropsychological basis of anxiety. In: Last C, Hersen M (eds). Handbook of Anxiety Disorders. NY Pergamon Press: 10–37.
100. Griez E, Lousberg H, van den Hout M (1987) CO2 vulnerability in panic disorder. Psychiatry Res 20: 87–95.
101. Grimaldi B, Fillion G (2000) 5-HT-moduline controls serotonergic activity: implication in neuroimmune reciprocal regulation mechanisms. Prog Neurobiol 60: 1–12.
102. Grove G, Coplan J, Hollander E (1997) The neuroanatomy of 5-HT dysregulation and panic disorder. J Neuropsych Clin Neurosci 9: 198–207.
103. Guilleminault C, Tharp B, Cousin D (1973) HVA and 5HIAA CSF measurements and 5HTP trials in some patients with involuntary movements. J Neurol Sci 18: 435–441.
104. Hamilton M (1959) The assessment of anxiety by rating. Br J Med Psychol 32, 50–55.
105. Hamilton S, Haghighi F, Heiman G, Klein D, Hodge S, Fyer A, Weissman M, Knowles J (2000a) Investigation of dopamine receptor (DRD4) and dopamine transporter (DAT) polymorphisms for genetic linkage or association to panic disorder. Am J Med Genet 96: 324–330.
106. Hamilton S, Heiman G, Haghighi F, Mick S, Klein D, Hodge S, Weissman M, Fyer A, Knowles J (1999) Lack of genetic linkage or association between a functional serotonin transporter polymorphism and panic disorder. Psychiatr Genet 9: 1–6.
107. Hamilton S, Slager S, Heiman G, Haghighi F, Klein D, Hodge S, Weissman M, Fyer A, Knowles J (2000b) No genetic linkage or association between a functional promoter polymorphism in the monoamine oxidase-A gene and panic disorder. Mol Psychiatry 5: 465–466.
108. Hamilton S, Slager S, Helleby L, Heiman G, Klein D, Hodge S, Weissman M, Fyer A, Knowles J (2001) No association or linkage between polymorphisms in the genes encoding cholecystokinin and the cholecystokinin B receptor and panic disorder. Mol Psychiatry 6: 59–65.
109. Han L, Nielsen D, Rosenthal N, Jefferson K, Kaye W, Murphy D, Altemus M, Humphries J, Cassano G, Rotondo A, Virkkunen M, Linnoila M, Goldman D (1999) No coding variant of the tryptophan hydroxylase gene detected in seasonal affective disorder, obsessive-compulsive disorder, anorexia nervosa, and alcoholism. Biol Psychiatry 45: 615–619.
110. Hariri A, Mattay V, Tessitore A, Kolachana B, Fera F, Goldman D, Egan M, Weinberger D (2002) Serotonin transporter genetic variation and the response of the human amygdala. Science 297: 400–403.
111. Hattori E, Yamada K, Toyota T, Yoshitsugu K, Toru M, Shibuya H, Yoshikawa T (2001) Association studies of the CT repeat polymorphism in the 5' upstream

region of the cholecystokinin B receptor gene with panic disorder and schizophrenia in Japanese subjects. Am J Med Genet 105: 779–782.
112. Hauser J, Leszczynska A, Samochowiec J, Czerski P, Ostapowicz A, Chlopocka M, Horodnicki J, Rybakowski J (2003) Association analysis of the insertion/deletion polymorphism in serotonin transporter gene in patients with affective disorder. Eur Psychiatry 18:129–132.
113. He M, Sibille E, Benjamin D, Toth M, Shippenberg T (2001) Differential effects of 5-HT1A receptor deletion upon basal and fluoxetine-evoked 5-HT concentrations as revealed by in vivo microdialysis. Brain Research 902: 11–17.
114. Heinz A, Jones D, Mazzanti C, Goldman D, Ragan P, Hommer D, Linnoila M, Weinberger D (2000) A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. Biol Psychiatry 47: 643–649.
115. Hensler J, Kovachich G, Frazer A (1991) A quantitative autoradiographic study of serotonin1A receptor regulation. Effect of 5,7-dihydroxytryptamine and antidepressant treatments. Neuropsychopharmacology 4: 131–144.
116. Hiltunen J, Åkerman K, Kuikka J, Bergström K, Halldin C, Nikula T, Räsänen P, Tiihonen J, Vauhkonen M, Karhu J, Kupila J, Länsimies E, Farde L (1998) Iodine-123 labeled nor-βas a potential tracer for serotonin transporter imaging in the human brain with single-photon emission tomography. Eur J Nucl Med 25: 19–23.
117. Inada Y, Yoneda H, Koh J, Sakai J, Himei A, Kinoshita Y, Akabame K, Hiraoka Y, Sakai T (2003) Positive association between panic disorder and polymorphism of the serotonin 2A receptor gene. Psychiatry Res 118: 25–31.
118. Ise K, Akiyoshi J, Horinouchi Y, Tsutsumi T, Isogawa K, Nagayama H (2003) Association between the CCK-A receptor gene and panic disorder. Am J Med Genet 118B: 29–31.
119. Ishiguro H, Arinami T, Yamada K, Otsuka Y, Toru M, Shibuya H (1997) An association study between a transcriptional polymorphism in the serotonin transporter gene and panic disorder in a Japanese population. Psychiatry Clin Neurosci 51: 333–335.
120. Iversen S (1984) 5-HT and anxiety. Neuropharmacology 23: 1553–1560.
121. Jacobs B, Azmitia E (1992) Structure and function of the brain serotonin system. Physiol Rev 72: 165–229.
122. Kahn R, Asnis G, Wetzler S, van Praag HM (1988a) Neuroendocrine evidence for serotonin receptor hypersensitivity in panic disorder. Psychopharmacologia 96: 360–364.
123. Kahn R, Westenberg H (1985) L-5-hydroxytryptophan in the treatment of anxiety disorders. J Affect Disord 8: 197–200.
124. Kahn R, Westenberg H, Verhoeven W, Gispen-de Wied C, Kamerbeek W (1987) 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.
125. Kahn R, Wetzler S, Asnis G, Kling M, Suckow R, van Praag H (1991) Pituitary hormone responses to meta-chlorophenylpiperazine in panic disorder and healthy control subjects. Psychiatry Res 37: 25–34.
126. Kahn R, Wetzler S, van Praag H, Asnis G, Strauman T (1988b) Behavioral indications for serotonin receptor hypersensitivity in panic disorder. Psychiatry Res 25: 101–104.

127. Katerndahl D, Realini J (1997) Quality of life and panic-related work disability in subjects with infrequent panic and panic disorder. J Clin Psychiatry 58: 153–158.
128. Kato T, Wang Z, Zoega T, Crowe R (1996) Missense mutation of the cholecystokinin B receptor gene: lack of association with panic disorder. Am J Med Genet 67: 401–405.
129. Kema I, de Vries E, Muskiet F (2000) Clinical chemistry of serotonin and metabolites. J Chromatogr B Biomed Sci Appl 747: 33–48.
130. Kendler K, Neale M, Kessler R, Heath A, Eaves L (1993) Panic disorder in women: a population-based twin study. Psychol Med 23: 397–406.
131. Kendler K, Prescott C, Myers J, NealeM (2003) The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. Arch Gen Psychiatry 60: 929–937.
132. Kennedy J, Bradwejn J, Koszycki D, King N, Crowe R, Vincent J, Fourie O (1999) Investigation of cholecystokinin system genes in panic disorder. Mol Psychiatry 4: 284–285.
133. Kent J, Coplan J, Martinez J, Karmally W, Papp L, Gorman J (1996) Ventilatory effects of tryptophan depletion in panic disorder: a preliminary report. Psychiatry Res 64: 83–90.
134. Kent J, Coplan J, Gorman J (1998) Clinical utility of the selective serotonin reuptake inhibitors in the spectrum of anxiety. Biol Psychiatry 44: 812–824.
135. Kessler R, Stang P, Wittchen H, Ustun T, Roy-Burne P, Walters E (1998) Lifetime panic-depression comorbidity in the National Comorbidity Survey. Arch Gen Psychiatry 55: 801–808.
136. Klaassen T, Klumperbeek J, Deutz N, van Praag H, Griez E (1998) Effects of tryptophan depletion on anxiety and on panic provoked by carbon dioxide challenge. Psychiatry Res 77: 167–174.
137. Klein DF (1993) False suffocation alarm, spontaneous panic and related conditions: An intergrative hypothesis. Arch Gen Psychiatry 50: 306–317.
138. Klein E, Zohar J, Geraci M, Murphy D, Uhde T (1991) Anxiogenic effects of m-CPP in patients with panic disorder: Comparison to caffeine's anxiogenic effects. Biol Psychiatry 30: 973–984.
139. Klerman G, Weissman M, Ouellette R, Johnson J, Greenwald S (1991) Panic attacks in the community. Social morbidity and health care utilization. J Am Med Assoc 265: 742–746.
140. Koszycki D, Zacharko R, Le Melledo J, Young S, Bradwejn J (1996) Effect of Acute Tryptophan Depletion on Behavioral, Cardiovascular, and Hormonal Sensitivity to Cholecystokinin-Tetrapeptide Challenge in Healthy Volunteers. Biol Psychiatry 40: 648–655.
141. Kuikka J, Pitkanen A, Lepola U, Partanen K, Vainio P, Bergstrom K, Wieler H, Kaiser K, Mittelbach L, Koponen et al (1995) Abnormal regional benzodiazepine receptor uptake in the prefrontal cortex in patients with panic disorder. Nucl Med Commun 16: 273–280.
142. Kuikka J, Tenhunen-Eskelinen M, Jurvelin J, Kiilianen H (1993) Physical performance of the Siemens MultiSPECT 3 gamma camera. Nucl Med Commun 14: 490–497.
143. Lanfumey L, Hamon M (2000) Central 5-HT(1A) receptors: regional distribution and functional characteristics. Nucl Med Biol 27: 429–435.

144. Lappalainen J, Dean M, Charbonneau L, Virkkunen M, Linnoila M, Goldman D (1995) Mapping of the serotonin 5-HT1D beta autoreceptor gene on chromosome 6 and direct analysis for sequence variants. Am J Med Genet 60: 157–161.
145. LeDoux JE (1994) The amygdala: contributions to fear and stress. Semin Neurosci 6: 231–237.
146. Lemonde S, Turecki G, Bakish D, Du L, Hrdina P, Bown C, Sequeira A, Kushwaha N, Morris S, Basak A, Ou X, Albert P (2003) Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J Neurosci 23: 8788–8799.
147. Lepola U, Nousiainen U, Puranen M, Riekkinen P, Rimon R (1990) EEG and CT findings in patients with panic disorder. Biol Psychiatry 28: 721–727.
148. Lerer B, Macciardi F, Segman R, Adolfsson R, Blackwood D, Blairy S, Del Favero J, Dikeos D, Kaneva R, Lilli R, Massat I, Milanova V, Muir W, Noethen M, Oruc L, Petrova T, Papadimitriou G, Rietschel M, Serretti A, Souery D, Van Gestel S, Van Broeckhoven C, Mendlewicz J (2001) Variability of 5-HT2C receptor cys23ser polymorphism among European populations and vulnerability to affective disorder. Mol Psychiatry 6: 579–585.
149. Lesch K, Bengel D, Heils A, Sabol S, Greenberg B, Petri S, Benjamin J, Muller C, Hamer D, Murphy D (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274: 1527–1531.
150. Lesch K, Mössner R (1998) Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? Biol Psychiatry 44: 179–192.
151. Liebowitz MR (1997) Panic disorder as a chronic illness. J Clin Psychiatry 58: 5–8.
152. Linnet K, Koed K, Wiborg O, Gregersen N (1995) Serotonin depletion decreases serotonin transporter mRNA levels in rat brain. Brain Research 697: 251–253.
153. Lira A, Zhou M, Castanon N, Ansorge M, Gordon J, Francis J, Bradley-Moore M, Lira J, Underwood M, Arango V (2003) Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. Biol Psychiatry 54: 960–971.
154. Logan J, Fowler J, Volkow N, Wolf A, Dewey S, Schlyer D, MacGregor R, Hitzemann R, Bendriem B, Gatley S, Christman D (1990) Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl]-(-)-cocaine PET studies in human subjects. J Cereb Blood Flow Metab 10: 740–747.
155. Lopez J, Liberzon I, Vazquez D, Young E, Watson S (1999) Serotonin1A receptor messenger RNA regulation in the hippocampus after acute stress. Biol Psychiatry 45: 934–937.
156. Lucki I (1998) The spectrum of behaviors influenced by serotonin. Biol Psychiatry 44: 151–162.
157. Maes M, Meltzer (1995) The serotonin hypothesis of major depression. In Bloom F. Kupfer D (eds). Psychopharmacology, the Fourth Generation of Progress. NY Raven Press: 933–944.
158. Maier W, Lichtermann D, Minges J, Oehrlein A, Franke P (1993) A controlled family study in panic disorder. J Psychiatr Res 27: 79–87.

159. Malison R, Price L, Berman R, van Dyck C, Pelton G, Carpenter L, Sanacora G, Owens M, Nemeroff C, Rajeevan N, Baldwin R, Seibyl J, Innis R, Charney D (1998) Reduced brain serotonin transporter availability in major depression as measured by [123I]-2 beta-carbomethoxy-3 beta-(4-iodophenyl)tropane and single photon emission computed tomography. Biol Psychiatry 44: 1090–1098.
160. Malizia A, Cunningham V, Bell C, Liddle P, Jones T, Nutt DJ (1998) Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. Arch Gen Psychiatry 55: 715–720.
161. Mamounas L, Mullen C, O'Hearn E, Molliver M (1991) Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. J Comp Neurol 314: 558–586.
162. Marazziti D, Rossi A, Dell'Osso L, Palego L, Placidi G, Giannaccini G, Lucacchini A, Cassano G (1999) Decreased platelet 3H-paroxetine binding in untreated panic disorder patients. Life Sci 65: 2735-2741.
163. Marek G, McDougle C, Price L, Seiden L (1992) A comparison of trazodone and fluoxetine: implications for a serotonergic mechanism of antidepressant action. Psychopharmacology 109: 2–11.
164. Maremmani I, Marini G, Fornai F (1998) Naltrexone-induced panic attacks. Am J Psychiatry 155: 447.
165. Maricq A, Peterson A, Brake A, Myers R, Julius D (1991) Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science 254: 432–437.
166. Marshall JR (1997) Alcohol and substance abuse in panic disorder. J Clin Psychiatry 58: 46–49.
167. Marteinsdottir I, Furmarkt T, Tillfors M, Agren H, Hartvig P, Fredrikson M, Langstrom B, Fischer H, Antoni G, Hagberg G (2001) Presynaptic serotonin imaging in social phobia using [3-11C]-5-hydroxy-L-tryptophan and PET. Neuro-Image 13: 1070.
168. Massat I, Souery D, Lipp O, Blairy S, Papadimitriou G, Dikeos D, Ackenheil M, Fuchshuber S, Hilger C, Kaneva R, Milanova V, Verheyen G, Raeymaekers P, Staner L, Oruc L, Jakovljevic M, Serretti A, Macciardi F, Van Broeckhoven C, Mendlewicz J (2000) A European multicenter association study of HTR2A receptor polymorphism in bipolar affective disorder. Am J Med Genet 96: 136–140.
169. Massot O, Rousselle J, Fillion M, Grimaldi B, Cloez-Tayarani I, Fugelli A, Prudhomme N, Seguin L, Rousseau B, Plantefol M, Hen R, Fillion G (1996) 5-hydroxytryptamine-moduline, a new endogenous cerebral peptide, controls the serotonergic activity via its specific interaction with 5-hydroxytryptamine1B/1D receptors. Mol Pharmacol 50: 752–762.
170. Matsushita S, Muramatsu T, Kimura M, Shirakawa O, Mita T, Nakai T, Higuchi S (1997) Serotonin transporter gene regulatory region polymorphism and panic disorder. Mol Psychiatry 2: 390–392.
171. Maura G, Thellung S, Andrioli G, Ruelle A, Raiteri M (1993) Release-regulating serotonin 5-HT1D autoreceptors in human cerebral cortex. J Neurochem 60: 1179–1182.
172. McCann U, Morgan C, Geraci M, Slate S, Murphy D, Post R (1997) Effects of the 5-HT3 antagonist, ondansetron, on the behavioral and physiological effects of

pentagastrin in patients with panic disorder and social phobia. Neuropsychopharmacology 17: 360–369.
173. Meltzer H, Maes M (1994) Effect of pindolol on the L-5-HTP-induced increase in plasma prolactin and cortisol concentrations in man. Psychopharmacologia 114: 635–643.
174. Meltzer M, Bastani M, Jayathilake M, Maes M (1997) Fluoxetine, but not Tricyclic Antidepressants, Potentiates the 5-Hydroxytryptophan-Mediated Increase in Plasma Cortisol and Prolactin Secretion in Subjects with Major Depression or with Obsessive Compulsive Disorder. Neuropsychopharmacology 17: 1–11.
175. Merikangas K, Stevens D, Fenton B, Stolar M, O'Malley S, Woods S, Risch N (1998) Co-morbidity and familial aggregation of alcoholism and anxiety disorders. Psychol Med 28: 773–788.
176. Meyer J, Kennedy S, Brown G (1998) No effect of depression on [(15)O]H2O PET response to intravenous d-fenfluramine. Am J Psychiatry 155: 1241–1246.
177. Meyer J, Swinson R, Kennedy S, Houle S, Brown G (2000) Increased left posterior parietal-temporal cortex activation after D-fenfluramine in women with panic disorder. Psychiatry Res 98: 133–143.
178. Middlemiss D, Bremer M, Smith S (1988) A pharmacological analysis of the 5-HT receptor mediating inhibition of 5-HT release in the guinea-pig frontal cortex. Eur J Pharmacol 157: 101–107.
179. Minov C, Baghai TC, Schule C, Zwanzger P, Schwarz M, Zill P, Rupprecht R, Bondy B (2001) Serotonin-2A-receptor and -transporter polymorphisms: lack of association in patients with major depression. Neurosci Lett 303: 119–122.
180. Moldin SO (2000) Neurobiology of anxiety and fear: challenges for genomic science of the new millennium. Biol Psychiatry 48: 1144–1146.
181. Mongeau R, Marsden C (1997) Effect of central and peripheral administrations of cholecystokinin-tetrapeptide on panic-like reactions induced by stimulation of the dorsal periaqueductal grey area in the rat. Biol Psychiatry 42: 335–344.
182. Moret C, Briley M (1997) 5-HT autoreceptors in the regulation of 5-HT release from guinea pig raphe nucleus and hypothalamus. Neuropharmacology 36: 1713–1723.
183. Mortimore C, Anderson I (2000) d-Fenfluramine in panic disorder: a dual role for 5-hydroxytryptamine. Psychopharmacologia 149: 251–258.
184. Nashold B, Wilson W, Slaughter D (1969) Sensations evoked by stimulation of the midbrain of man. J Neurosurg 30:14 –24.
185. Neumeister A, Bain E, Nugent A, Carson RE, Bonne O, Luckenbaugh D, Eckelman W, Herscovitch P, Charney D, Drevets W (2004) Reduced serotonin type 1A receptor binding in panic disorder. J Neurosci 24: 589–591.
186. Nielsen D, Jenkins G, Stefanisko K, Jefferson K, Goldman D (1997) Sequence, splice site and population frequency distribution analyses of the polymorphic human tryptophan hydroxylase intron 7. Mol Brain Res 45:145–148.
187. Nishizawa S, Benkelfat C, Young S, Leyton M, Mzengeza S, de Montigny C, Blier P, Diksic M (1997) Differences between males and females in rates of serotonin synthesis in human brain. Proc Natl Acad Sci U S A 94: 5308–5313.
188. Nordahl T, Stein M, Benkelfat C, Semple W, Andreason P, Zametkin A, Uhde T, Cohen R (1998) Regional cerebral metabolic asymmetries replicated in an independent group of patients with panic disorders. Biol Psychiatry 44: 998–1006.

189. Noyes R, Crowe R, Harris E, Hamra B, McChesney C, Chaudhry D (1986) Relationship between panic disorder and agoraphobia. A family study. Arch Gen Psychiatry 43: 227–232.
190. Noyes R, Reich J, Christiansen J, Suelzer M, Pfohl B, Coryell W (1990) Outcome of panic disorder. Relationship to diagnostic subtypes and comorbidity. Arch Gen Psychiatry 47: 809–818.
191. Nutt DJ (1986) Increased central alpha 2-adrenoceptor sensitivity in panic disorder. Psychopharmacologia 90: 268–269.
192. Nutt DJ (1998) Antidepressants in panic disorder: clinical and preclinical mechanisms. J Clin Psychiatry 59: 24–28.
193. Nutt DJ, Glue P, Lawson C, Wilson S (1990) Flumazenil provocation of panic attacks. Evidence for altered benzodiazepine receptor sensitivity in panic disorder. Arch Gen Psychiatry 47: 917–925.
194. Nutt DJ, Nash J, Bell C, Adrover M, Sargent P, Rabiner E, Hood S, Argyropoulos S, Grasby P (2003) Antidepressant mechanisms in anxiety disorders. Eur Neuropsychopharmacol 13: 173.
195. Nutt DJ, Forshall S, Bell C, Rich A, Sandford J, Nash J, Argyropoulos S (1999) Mechanisms of action of selective serotonin reuptake inhibitors in the treatment of psychiatric disorders. Eur Neuropsychopharmacol 9: 81–86.
196. Ohara K, Nagai M, Suzuki Y, Ochiai M, Ohara K (1998) No association between anxiety disorders and catechol-O-methyltransferase polymorphism. Psychiatry Res 80: 145–148.
197. Ontiveros A, Fontaine R, Breton G, Elie R, Fontaine S, Dery R (1989) Correlation of severity of panic disorder and neuroanatomical changes on magnetic resonance imaging. J Neuropsychiatry Clin Neurosci 1: 404–408.
198. Oswald P, Souery D, Massat I, Del Favero J, Linotte S, Papadimitriou G, Dikeos D, Kaneva R, Milanova V, Oruc L, Ivezic S, Serretti A, Lilli R, Van Broeckhoven C, Mendlewicz J (2003) Lack of association between the 5HT2A receptor polymorphism (T102C) and unipolar affective disorder in a multicentric European study. Eur Neuropsychopharmacol 13: 365–368.
199. Parks C, Robinson P, Sibille E, Shenk T, Toth M (1998) Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci U S A 95: 10734–10739.
200. Parsian A, Cloninger C (2001) Serotonergic pathway genes and subtypes of alcoholism: association studies. Psychiatr Genet 11: 89–94.
201. Pelissolo A, Lepine JP (1998) Acute anxiety attack. Diagnostic orientation and management in an emergency situation. Rev Prat 48: 1029–1032.
202. Perna G, Bertani A, Caldirola D, Bellodi L (1996) Family history of panic disorder and hypersensitivity to CO2 in patients with panic disorder. Am J Psychiatry 153: 1060–1064.
203. 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.
204. Perna G, Bella D, Favaron E, Cucchi M, Liperi L, Bellodi L (2004) Lack of relationship between CO(2) reactivity and serotonin transporter gene regulatory region polymorphism in panic disorder. Am J Med Genet 129: 41–43.

205. Pitchot W, Ansseau M, Gonzalez Moreno A, Hansenne M, von Frenckell R (1992) Dopaminergic function in panic disorder: Comparison with major and minor depression. Biol Psychiatry 32: 1004–1011.
206. Pitts F, McClure J (1967) Lactate metabolism in anxiety neurosis. N Engl J Med 277: 1329–1336.
207. Pohl R, Yeragani V, Balon R, Lycaki H, McBride R (1992) Smoking in patients with panic disorder. Psychiatry Res 43: 253–262.
208. Pollack M, Otto M (1997) Long-term course and outcome of panic disorder. J Clin Psychiatry 58: 57–60.
209. Pranzatelli M, Durkin M, Barkai A (1994) Quantitative autoradiography of 5-hydroxytryptaminelA binding sites in rats with chronic neonatal 5,7-dihydroxytryptamine lesions. Dev Brain Res 80: 1–6.
210. Pyke R, Greenberg H (1986) Norepinephrine challenges in panic patients. J Clin Psychopharmacol 6: 279–285.
211. Ramboz S, Oosting R, Amara D, Kung H, Blier P, Mendelsohn M, Mann J, Brunner D, Hen R (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci U S A 95: 14476–14481.
212. Ramos A, Tagliaferro P, Lopez E, Pecci Saavedra J, Brusco A (2000) Neuroglial interactions in a model of para-chlorophenylalanine-induced serotonin depletion. Brain Res 883: 1–14.
213. Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86: 284–295.
214. Redmond D, Huang Y (1979) Current concepts. II. New evidence for a locus coeruleus-norepinephrine connection with anxiety. Life Sci 25: 2149–2162.
215. Rees M, Norton N, Jones I, McCandless F, Scourfield J, Holmans P, Moorhead S, Feldman E, Sadler S, Cole T, Redman K, Farmer A, McGuffin P, Owen M, Craddock N (1997) Association studies of bipolar disorder at the human serotonin transporter gene (hSERT; 5HTT). Mol Psychiatry 2: 398–402.
216. Regier D, Narrow W, Rae D (1990) The epidemiology of anxiety disorders: the Epidemiologic Catchment Area (ECA) experience. J Psychiatr Res 24: 3–14.
217. Reiman E (1987) The study of panic disorder using positron emission tomography. Psychiatr Dev 5: 63–78.
218. Rothe C, Gutknecht L, Freitag C, Tauber R, Mössner R, Franke P, Fritze J, Wagner G, Peikert G, Wenda B, Sand P, Jacob C, Rietschel M, Nöthen M, Garritsen H, Fimmers R, Deckert J, Lesch K (2004) Association of a functional -1019C>G 5-HT1A receptor gene polymorphism with panic disorder with agoraphobia. Int J Neuropsychopharmacol 7: 189–192.
219. Sabol S, Hu S, Hamer D (1998) A functional polymorphism in the monoamine oxidase A gene promoter. Hum Genet 103: 273–279.
220. Sargent P, Kjaer K, Bench C, Rabiner E, Messa C, Meyer J, Gunn R, Grasby P, Cowen P (2000) Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. Arch Gen Psychiatry 57: 174–180.
221. Schlegel S, Steinert H, Bockisch A, Hahn K, Schloesser R, Benkert O (1994) Decreased benzodiazepine receptor binding in panic disorder measured by IOMAZENIL-SPECT. A preliminary report. Eur Arch Psychiatry Clin Neurosci 244: 49–51.

222. Schmidt N, Storey J, Greenberg B, Santiago H, Li Q, Murphy D (2000) Evaluating gene x psychological risk factor effects in the pathogenesis of anxiety: a new model approach. J Abnorm Psychol 109: 308–320.
223. Schreiber R, De Vry J (1993) 5-HT1A receptor ligands in animal models of anxiety, impulsivity and depression: Multiple mechanisms of action? Prog Neuropsychopharmacol Biol Psychiatry 17: 87–104.
224. Schruers K, Klaassen T, Pols H, Overbeek T, Deutz N, Griez E (2000) Effects of tryptophan depletion on carbon dioxide provoked panic in panic disorder patients. Psychiatry Res 93: 179–187.
225. Schruers K, van Diest R, Overbeek T, Griez E (2002) Acute –5-hydroxytryptophan administration inhibits carbon dioxide-induced panic in panic disorder patients. Psychiatry Res 113: 237–243.
226. Selbie L, Hill S (1998) G protein-coupled-receptor cross-talk: the fine-tuning of multiple receptor-signalling pathways. Trends Pharmacol Sci 19: 87–93.
227. Shear M, Rucci P, Williams J, Frank E, Grochocinski V, Vander Bilt J, Houck P, Wang T (2001) Reliability and validity of the Panic Disorder Severity Scale: replication and extension. J Psychiatr Res 35: 293–296.
228. Sheehan D, Lecrubier Y, Sheehan K, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar G (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59: 22–33.
229. Shlik J, Vasar E, Bradwejn J (1997a) Cholecystokinin and psychiatric disorders: role in aetiology and potential of receptor antagonists in therapy. CNS Drugs 8: 134–152.
230. Shlik J, Vasar V, Aluoja A, Kingisepp P, Jagomagi K, Vasar E, Rago L, Bradwejn J (1997b) The Effect of Cholecystokinin Tetrapeptide on Respiratory Resistance in Healthy Volunteers. Biol Psychiatry 42: 206–212.
231. Shlik J, Aluoja A, Vasar V, Vasar E, Podar T, Bradwejn J (1997c) 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.
232. Solyom L (1994) Controlling panic attacks with fenfluramine. Am J Psychiatry 151: 621–622.
233. Spurlock G, Buckland P, O'Donovan M, McGuffin P (1994) Lack of effect of antidepressant drugs on the levels of mRNAs encoding serotonergic receptors, synthetic enzymes and 5HT transporter. Neuropharmacology 33: 433–440.
234. Stein M, Uhde T (1989) Infrequent occurrence of EEG abnormalities in panic disorder. Am J Psychiatry 146: 517–520.
235. Ströhle A, Ehrenthal H, Holsboer F (2001) Increased nocebo response and CCK-4-hypersensitivity in female patients with panic disorder. Pharmacopsychiatry 34: 203.
236. Stutzmann G, LeDoux J (1999) GABAergic antagonists block the inhibitory effects of serotonin in the lateral amygdala: a mechanism for modulation of sensory inputs related to fear conditioning. J Neurosci 19: 8.
237. Tadic A, Rujescu D, Szegedi A, Giegling I, Singer P, Moller H, Dahmen N (2003) Association of a MAOA gene variant with generalized anxiety disorder, but not with panic disorder or major depression. Am J Med Genet 117: 1–6.

238. Tammela L, Rissanen A, Kuikka J, Karhunen L, Bergstrom K, Repo-Tiihonen E, Naukkarinen H, Vanninen E, Tiihonen J, Uusitupa M (2003) Psychopharmacology, 170: 89–93.
239. Targum S, Marshall L (1989a) Fenfluramine provocation of anxiety in patients with panic disorder. Psychiatry Res 28: 295–306.
240. Taylor S, Koch W, McNally R, Crockett D (1992) Conceptualisations of anxiety sensitivity. Psychol Assess 4: 245–250.
241. Thorpe L, Westlund K, Kochersperger L, Abell C, Denney R (1987) Immunocytochemical localization of monoamine oxidases A and B in human peripheral tissues and brain. J Histochem Cytochem 35: 23–32.
242. Tregouet D, Barbaux S, Escolano S, Tahri N, Golmard J, Tiret L, Cambien F (2002) Specific haplotypes of the P-selectin gene are associated with myocardial infarction. Hum Mol Gen 11: 2015–2023.
243. Tweed J, Schoenbach V, George L, Blazer D (1989) The effects of childhood parental death and divorce on six-month history of anxiety disorders. Br J Psychiatry 154: 823–828.
244. Tyrer P, Shawcross C (1988) Monoamine oxidase inhibitors in anxiety disorders. J Psychiatr Res 22: 87–98.
245. Tõnisson N, Zernant J, Kurg A, Pavel H, Slavin G, Roomere H, Meiel A, Hainaut P, Metspalu A (2002) Evaluating the arrayed primer extension resequencing assay of TP53 tumor suppressor gene. Proc Natl Acad Sci USA 99: 5503–5508.
246. Törk I, Hornung J (1990) Raphe Nuclei and the Serotonergic System. The Human Nervous System in the Human Nervous system. Paxinos G, Orlando F (eds). Academic Press: 1001–1022.
247. van Megen H, Westenberg H, den Boer J, Slaap B, Scheepmakers A (1997) Effect of the selective serotonin reuptake inhibitor fluvoxamine on CCK-4 induced panic attacks. Psychopharmacologia 129: 357–364.
248. van Praag H, Lemus C, Kahn R (1987) Hormonal probes of central serotonergic activity: do they really exist? Biol Psychiatry 22: 86–98.
249. Verburg K, Griez E, Meijer J, Pols H (1995) Respiratory disorders as a possible predisposing factor for panic disorder. J Affect Disord 33: 129–134.
250. Verge D, Daval G, Marcinkiewicz M, Patey A, Mestikawy S, Gozlan H, Hamon M (1986) Quantitative autoradiography of multiple 5-HT1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. J Neurosci 6: 3474–3482.
251. Vythilingam M, Anderson E, Goddard A, Woods S, Staib L, Charney D, Bremner J (2000) Temporal lobe volume in panic disorder – a quantitative magnetic resonance imaging study. Psychiatry Res 99: 75–82.
252. Wang Z, Valdes J, Noyes R, Zoega T, Crowe R (1998) Possible association of a cholecystokinin promotor polymorphism (CCK-36CT) with panic disorder. Am J Med Genet 81: 228–234.
253. Weissman M, Bland R, Canino G, Faravelli C, Greenwald S, Hwu H, Joyce P, Karam E, Lee C, Lellouch J, Lepine J, Newman S, Oakley-Browne M, Rubio-Stipec M, Wells J, Wickramaratne P, Wittchen H, Yeh E (1997) The cross-national epidemiology of panic disorder. Arch Gen Psychiatry 54: 305–309.
254. Welner S, De Montigny C, Desroches J, Desjardins P, Suranyi-Cadotte B (1989) Autoradiographic quantification of serotonin1A receptors in rat brain following antidepressant drug treatment. Synapse 4: 347–352.

255. Wetzler S, Asnis G, DeLecuona J, Kalus O (1996) Serotonin function in panic disorder: intravenous administration of meta-chlorophenylpiperazine. Psychiatry Res 64: 77–82.
256. Weyler W, Hsu Y, Breakefield X (1990) Biochemistry and genetics of monoamine oxidase. Pharmacol Ther 47: 391–417.
257. Willeit M, Praschak-Rieder N, Neumeister A, Pirker W, Asenbaum S, Vitouch O, Tauscher J, Hilger E, Stastny J, Brucke T, Kasper S (2000) [123I]-beta-CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. Biol Psychiatry 47: 482–489.
258. Williams J, Spulock G, McGuffin P, Mallet J, Nöthen M, Aschauer H, Nylander P, Macciardi F, Owen M (1996) Association between schizophrenia and T102C polymorphism of the 5 hydroxytryptamine type 2a-receptor. Lancet 347: 1294–1296.
259. Wittchen H, Essau C (1993) Epidemiology of panic disorder: progress and unresolved issues. J Psychiatr Research 27: 47–68.
260. Woo J, Yoon K, Yu B (2002) Catechol O-methyltransferase genetic polymorphism in panic disorder. Am J Psychiatry 159: 1785–1787.
261. Yamada K, Hattori E, Shimizu M, Sugaya A, Shibuya H, Yoshikawa T (2001) Association studies of the cholecystokinin B receptor and A2a adenosine receptor genes in panic disorder. J Neural Transm Gen Sec 108: 837–848.
262. Zandbergen J, Bright M, Pols H, Fernandez I, de Loof C, Griez E (1991) Higher lifetime prevalence of respiratory diseases in panic disorder? Am J Psychiatry 148: 1583–1585.
263. Zaubler T, Katon W (1998) Panic disorder in the general medical setting. J Psychosom Res 44: 25–42.
264. Zhang X, Beaulieu J, Sotnikova T, Gainetdinov R, Caron M (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science 305: 217.
265. Zifa E, Fillion G (1992) 5-Hydroxytryptamine receptors. Pharmacol Rev 44: 401–458.

SUMMARY IN ESTONIAN

Serotoniini funktsioon paanikahäire korral: kliinilistest eksperimentidest aju piltdiagnostika ja geeniuuringuteni

Kokkuvõte

Paanikahäire on levinud ja olulise raskusastmega tervisehäire, mille etiopatogeneesi seostatakse mitme neurokeemilise süsteemiga. Seni teostatud kliinilised ja eksperimentaalsed uuringud viitavad serotoniini (5-HT) süsteemi tähtsusele paanikahäire tekkes, kuid selle täpne roll on ebaselge (Coplan et al., 1992). On püstitatud kaks tähenduselt vastandlikku hüpoteesi, mis seletavad 5-HT süsteemi osatähtsust paanikahäire tekkes. Vastavalt 5-HT liigtalitluse hüpoteesile (Iversen, 1984), paanikahäire puhul on tegemist 5-HT süsteemi ülemäärase aktiivsusega või postsünaptiliste retseptorite ülitundlikkusega. 5-HT alatalitluse hüpoteesi kohaselt (Deakin and Graeff, 1991; Eriksson, 1993) 5-HT süsteem omab parssivat mõju paanikahoogude tekkele ja paanikahäire tekkimist seejuures seletatakse 5-HT süsteemi puudega teatud ajupiirkondades.

Uurimistöö eesmärgid

Käesoleva töö uldeesmärgiks oli 5-HT süsteemi funktsionaalse rolli uurimine paanikahäire patogeneesis ja neurobioloogias, kasutades eksperimentaalseid, isotoopdiagnostilisi ning geneetilisi meetodeid.
Uuringute eesmärgid olid järgmised:

1. Uurida 5-HTP manustamisest tingitud 5-HT sisalduse tõusu mõju ajus CCK-4 panikogeensele toimele tervetel isikutel (I).
2. Uurida 5-HT süsteemi geenide funktsionaalsete polümorfismide mõju CCK-4- provotseeritud paanikahoogude sagedusele tervetel isikutel (II).
3. Määrata 5-HT transporteri sidumisomadusi ajus paanikahäirega ja generaliseerunud ärevushäirega patsientidel võrrdluses tervete isikutega (III, IV).
4. Selgitada 5-HT süsteemi geneetilise regulatsiooni osa paanikahäire puhul, uurides võimalikke seoseid 5-HT süsteemi geneetilise polumorfismi ja paanikahäire fenotüüpide vahel (V, VI).

Uuringute meetodid

Uuringutesse (I, II) oli kaasatud 32 tervet vabatahtlikku meest ja naist ajalehekuulutuste kaudu. Uuringuprotseduur teostati kahes paralleelgrupis kaksikpimedal meetodil. Juhuvaliku korras said pooled osalejad 90 minutit enne CCK-4

(50 μg) veenisiseselt manustamist 5-HTP (Tript-OH, 200 mg) ja pooled platseebot kapslites. Uuritavate enesetunde muutusi ja reaktsiooni CCK-4 manustamisele hinnati visuaalsete analoogskaalade ja paanikasümptomite skaala abil ning neil jälgiti püsivalt südamelöökide sagedust ja arteriaalset vererõhku. 5-HT transporteri ajutalitlust uuriti 8 paanikahäirega patsiendil, 8 remissioonis paanikahäirega patsiendil (III) ning 7 generaliseerunud ärevushäirega patsiendil (IV), kasutades nor-β-CIT radioligandi ja SPECT meetodit ning võrreldes tervete isikutega. 5-HT süsteemi seoste uurimisel geneetiliste polümorfismidega osales 158 paanikahäirega patsienti (80% naist, keskmine vanus 38,0 aastat) koos või ilma agorafoobiata ning 215 tervet vabatahtlikku isikut (74% naist, keskmine vanus 39,8 aastat) (V). Teises assotsiatsiooniuuringus (VI) uuriti APEX-meetodil 90 ühenukleotiidset polümorfismi 21 geenist 127 paanikahäirega patsiendil (82% olid naised, keskmine vanus 38,4 aastat) ning 146 tervetel inimestel (75% olid naised, keskmine vanus 38,7 aastat). Paanikahäire ning teiste psüühikahäirete olemasolu tõendati või välistati arstlikul uurimisel ning rahvusvahelise neuropsühhiaatrilise MINI intervjuu läbiviimisel.

Tulemused

Esimene uuring näitas, et 5-HTP manustamisest tingitud 5-HT sisalduse tõus ajus vähendas oluliselt CCK-4 poolt provotseeritud paanikahoogude sagedust ning kognitiivseid sümptomeid naistel ning nõrgestas paaanikahoo somaatilisi sümptomeid tervetel meestel. Teises uuringus leiti, et CCK-4 poolt esilekutsutud paanikahoogude sagedus on oluliselt väiksem monoamiini oksüdaas A geeni kõrge aktiivsusega polümorfismidega kui funktsionaalselt madala aktiivsusega polümorfismidega tervetel. Lisaks oli CCK-4 paanikahoogude sagedus oluliselt väiksem naistel monoamiini oksüdaas A geeni kõrge aktiivsusega või 5-HT transporteri geeni madala aktiivsusega polümorfismide korral. Seejuures ei leitud seost paanikahoogude sageduse ning uuritud 5-HT süsteemi geneetiliste polümorfismide vahel meestel. Radioisotoopdiagnostilised uuringud näitasid, et võrreldes tervete isikutega oli aktiivse paanikahäirega patsientidel 5-HT transporteri sisaldus alanenud keskajus, talamuses ja oimusagarates. Samal ajal oli remissioonis paanikahäirega patsientidel 5-HT transporteri sisaldus normis keskajus ja oimusagarates ning osutus oluliselt madalamaks talamuses. Generaliseerunud ärevushäirega patsientidel ei leitud muutusi 5-HT-transporteris võrrelduna tervete isikutega. Geneetilise polümorfismi analüüs näitas, et võrreldes kontrollrühmaga ilmnes paanikahäirega patsientidel oluline seos 5-HT transporteri geeni funktsionaalselt kõrge aktiivsusega polümorfismiga. Lisaks monoamiini oksüdaas A geeni kõrge aktiivsusega variandid olid paanikahäirega naistel sagedasemad kui tervetel naistel. Teiselt poolt, mittefunktsionaalsed 5-HT süsteemi geenide polümorfismid, nagu 5-HT transporteri VNTR, trüptofaani hüdroksülaasi 218A/C ja 5-HT1B retseptori 861G/C, ei näidanud selget seost paanikahäire fenotüübiga. Teise assotsiatsiooniuuringu tulemusena leiti, et

paanikahäire fenotüübiga on spetsiifiliselt seotud 5-HT2A-retseptori ning dopamiini-1-retseptori polümorfismid. Kaasuva unipolaarse depressiooniga paanikahäirega patsientide või kogu paanikahäire rühma võrdlemisel tervete inimestega leiti olulised seosed CCK süsteemi ning 5-HT1A- ja 5-HT2C-retseptorite geenide polümorfismidega.

Järeldused

1. 5-HTP manustamine vähendas oluliselt CCK-4 poolt esilekutsutud paanikahoogude sagedust ja kognitiivseid sümptomeid naistel ning vähendas paanikahoo somaatilisi sümptomeid tervetel meestel.
2. CCK-4 poolt põhjustatud paanikahoogude sagedus oli seotud monoamiini oksüdaas A ning 5-HT transporteri geenide promootor regiooni funktsionaalsete polümorfismidega tervetel naistel, kuid mitte meestel. Need tulemused toetavad hüpoteesi 5-HT spetsiifilisest pärssivast toimest paanikahoo sümptomitele ja viitavad edasiste uuringute vajadusele, selgitamaks soost sõltuvaid 5-HT mõjusid paanikahäire neurobioloogias.
3. Paanikahäirega patsientidel on leitud kliinilisest seisundist sõltuv 5-HT transporteri sisalduse langus, mis võib peegeldada 5-HT närviülekande defitsiiti või kompensatoorset protsessi 5-HT süsteemis, mille eesmärk on tõsta 5-HT sünaptilist taset. 5-HT transporteri sidumine ajus ei olnud muutunud generaliseerunud ärevushäirega patsientidel, mis võib tähendada, et 5-HT transporter omab erinevat funktsionaalset rolli paanikahäire ja generaliseerunud ärevushäire korral.
4. Paanikahäirega patsientidel leiti, et 5-HT transporteri funktsionaalselt kõrge aktiivsusega polümorfism esineb oluliselt sagedamini võrreldes kontrollrühmaga, kusjuures mitte-funktsionaalsed polümorfismid, nagu 5-HT transporteri VNTR, trüptofaani hüdroksülaasi 218A/C ja 5-HT1B retseptori 861G/C, ei olnud seotud paanikahäire fenotüübiga. Samuti leiti, et paanikahäirega naistel koos agorafoobiaga oli monoamiini oksüdaas A geeni kõrge aktiivsusega variatsiooni esinemine sagedasem võrreldes tervete naistega. Need tulemused viitavad sellele, et geneetilised polümorfismid, mis on seotud madala närviülekandega 5-HT süsteemis võivad osaleda paanikahäire tekkes.
5. Mitmete erinevate kandidaatgeenide skriining tõi esile, et 5-HT süsteemi teatud geneetilised polümorfismid, nagu 5-HT2A retseptori 102T-C variatsioon, võivad omada spetsiifilist rolli kõrgenenud vastuvõtlikkuses paanikahäirele ja 5-HT2C retseptori Cys23Ser ning 5-HT1A retseptori 1018C-G polümorfismid on tõenäolisemalt enam seotud meeleoluhäirega kaasnevate paanikahäire fenotüüpidega.

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PUBLICATIONS

E. Maron, I. Tõru, V. Vasar, J. Shlik. The effect of 5-hydroxytryptophan on cholecystokinin-4-induced panic attacks in healthy volunteers. Journal of Psychopharmacology (2004) 18 (2): 194–199.

E. Maron, G. Tasa, I. Tõru, A. Lang, V. Vasar, J. Shlik. Association between serotonin-related genetic polymorphisms and CCK-4-induced panic attacks with or without 5-hydroxytryptophan pretreatment in healthy volunteers. Word Journal of Biological Psychiatry (2004) 5 (3): 149–154.

E. Maron, J. T. Kuikka, J. Shlik, V. Vasar, E. Vanninen, J. Tiihonen. Reduced brain serotonin transporter binding in patients with panic disorder. Psychiatry Research: Neuroimaging (in press)

E. Maron, J. T. Kuikka, K. Ulst, J. Tiihonen, V. Vasar, J. Shlik. SPECT imaging of serotonin transporter binding in patients with generalized anxiety disorder. European Archives of Psychiatry and Clinical Neuroscience (in press)

E. Maron, A. Lang, G. Tasa, L. Liivlaid, I. Tõru, A. Must, V. Vasar, J. Shlik. Associations between serotonin related gene polymorphisms and panic disorder. Submitted to International Journal of Neuropsychopharmacology

E. Maron, T. Nikopensius, S. Kõks, S. Altmäe, E. Heinaste, K. Vabrit, V. Tammekivi, P. Hallast, K. Koido, A. Kurg, A. Metspalu, E. Vasar, V. Vasar, J. Shlik. Association study of 90 candidate genetic polymorphisms in panic disorder. Psychiatric Genetics (in press)

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