PhD thesis

Relating cerebral serotonin 2A receptor and serotonin transporter binding to personality and familial risk for mood disorder

Vibe Gedsø Frøkjær, MD
Neurobiology Research Unit
Copenhagen University Hospital
Rigshospitalet 2008

Supervisors

Gitte Moos Knudsen, MD, professor, DMSc, Neurobiological Research Unit, Department of Neurology, Copenhagen University Hospital Rigshospitalet

Lars Vedel Kessing, MD, professor DMSc, Department of Psychiatry, Copenhagen University Hospital Rigshospitalet

Steen Gregers Hasselbalch, MD, DMSc, associate professor, Neurobiological Research Unit, Department of Neurology, Copenhagen University Hospital Rigshospitalet

Evaluating committee

Jarmo Hietala, MD, professor, Department of Pyschiatry, University of Turku, Finland

Jeffrey Meyer, MD, professor, Centre for Addiction and Mental Health, University of Toronto, Canada

Gunhild Waldemar (committee chairman), MD, professor, Department of Neurology, Copenhagen University Hospital, Rigshospitalet, Denmark

The thesis was handed in January 2th 2008, accepted for oral defense April 21th 2008, and defended June 11th 2008

Front page illustration:
A happy twin and a sad twin both rollerskating / Elton Engkilde

Acknowledgements

I remain grateful to the volunteers who generously participated in these studies.

I thank all my colleagues at Neurobiology Research Unit and my co-authors from other institutions for inspiring collaborations. In particular, I thank my closest colleagues, David Erritzøe, Maj Vinberg, Claus Svarer, Lars Pinborg, Steven Haugbøl, William Baaré, and Robin de Nijs, with whom I could simply not have worked without and my fellow PhD students Lisbeth Marner, Karine Madsen and Jan Kalbitzer for inspiring and enjoyable collaboration. I thank Gerda Thomsen, Karin Stahr, Anita Dole, Kirsten Hornshyld, Bente Høy, and the staff at the PET and Cyclotron Unit, Rigshospitalet, for taking very good care of the study participants, for always superb technical assistance, and for personal support. I thank Klaus Holst and Esben Budtz-Jørgensen for expert advice and discussion of statistical tools. I am grateful to have not only bright and skilled colleagues around me but, importantly, also good friends.

I am most thankful for excellent supervision and always patient guidance from Gitte Moos Knudsen who supervised this thesis in its whole, Lars Vedel Kessing who supervised the work connected to familial risk of developing mood disorders, Erik Lykke Mortensen who supervised the personality related aspects, and Steen Hasselbalch who supervised general methodological aspects.

I thank the Faculty of Health Sciences, Copenhagen University, The Lundbeck Foundation and Rigshospitalet for financial support of the studies.

Finally I am happy to be able to thank my entire family for supporting me during my study period, especially, my parents, Erik Frøkjær and Lisbeth Gedsø, and my husbond, August Engkilde, and our two kids, Elton and Ella Rebekka, who through their love, awareness, and care have been my basic source of energy and inspiration.

Vibe Gedsø Frøkjær, Nærum, January 2th 2008

Summary

This thesis focuses on pre- and postsynaptic characteristics of serotonergic neurotransmission in the living human brain and their relation to personality and familial risk factors for mood disorders.

Mood disorders are common with a life time risk of major depression of at least 10% being twice as common in females than in males. The most potent risk factor for developing a mood disorder is a family history of mood disorder, but the specific factors mediating the effects are not well understood. Disadvantageous combinations of genetic profile, environmental stress factors, and disturbances in serotonergic neurotransmission seem to be important in the pathophysiology. Concerning the disturbances in serotonergic neurotransmission, it is not clear whether they represent trait or state markers of mood disorders.

The thesis is based on 3 studies that aim at exploring possible trait characteristics of mood disorders by linking personality and familial risk factors and brain biology in terms of serotonin 2A receptor and serotonin transporter binding and distribution. In study 1, we examined the possible association between the personality risk factor, neuroticism, and serotonin 2A receptor binding in regions of relevance to personality and mood disorders in a large sample of healthy volunteers. We identified a link between high neuroticism and high frontolimbic serotonin 2A receptor binding. Based on these findings, we hypothesized that this link might be mediated by genetic risk factors for mood disorders. Further, we hypothesized that the serotonin transporter binding in brain regions relevant to mood disorders might also represent a trait characteristic of mood disorder. These hypotheses were explored in a high-risk low-risk study design comparing twins at high familial risk with twins at very low risk of developing mood disorders. We found a stronger coupling between neuroticism and frontolimbic serotonin 2A receptor binding in individuals at high than at low familial risk of mood disorder (study 2). Also, we found a lower SERT binding in dorsolateral prefrontal cortex and anterior cingulate in individuals at high familial risk (study 3).

In conclusion, the results generated from this work suggest that alterations in the serotonergic neurotransmission may represent a trait marker of mood disorders that could either be established during early brain development and/or compensatory

adaptations to low synaptic serotonin. Thus our findings offer a neurobiological link between personality and familial risk factors and mood disorders. This may guide future longitudinal studies aiming at elucidating which characteristics of serotonergic neurotransmission are typical for at-risk individuals who develop mood disorder.

Dansk resumé

Afhandlingen relaterer hjernens serotonerge transmittersystem til personlighed og familiær risiko for udvikling af affektiv sygdom.

Affektive sygdomme er hyppige. Livstidsrisikoen for at udvikle depression er mindst 10 %, og depression optræder dobbelt så hyppig hos kvinder som hos mænd. Den stærkeste risikofaktor for udvikling af affektiv sygdom er at have en førstegradsslægtning med sygdommen, men det er uklart præcis hvad der betinger, at man rent faktisk udvikler en affektiv sygdom. Ufordelagtige kombinationer af genetik, miljømæssige stressfaktorer samt forstyrrelser i den serotonerge neurotransmission synes alle at spille en vigtig rolle i patofysiologien. I en række studier har man tidligere fundet abnormiteter i det serotonerge system i hjernen hos depressive patienter, men det er usikkert om dette repræsenterer en risikofaktor for udvikling af affektiv sygdom eller om det snarere bør fortolkes som en konsekvens af sygdomsprocessen.

Afhandlingen er baseret på 3 studier der relaterer personlighedsmæssige og familiære risikofaktorer for affektiv sygdom til serotonin 2A receptor- og serotonintransporter-bindingen i hjernen. I det første studie undersøgte vi sammenhængen mellem "neuroticisme", en personlighedskomponent som er en kendt risikofaktor for udvikling af depression, og serotonin 2A receptor-bindingen i de hjerneområder, der vides at være involveret ved personlighed og affektiv sygdom. Dette studie var baseret på hjerneskanninger fra 83 raske frivillige personer. Vi fandt en positiv sammenhæng mellem en neuroticisme-score og serotonin 2A receptor bindingen i frontolimbiske regioner. På denne baggrund opstillede vi hypotesen, at sammenhængen kunne være genereret af genetiske risikofaktorer for affektiv sygdom. Ydermere opstillede vi hypotesen at serotonintransporter-bindingen ligeledes ville være ændret hos individer med høj familiær risiko for at udvikle affektiv sygdom i relevante hjerneområder. Disse hypoteser blev testet ved at sammenligne en gruppe tvillinger med høj familiær risiko for at udvikle affektiv lidelse med en gruppe tvillinger med lav familiær risiko. Vi fandt en mere udtalt kobling mellem neuroticisme og frontolimbisk serotonin 2A receptor binding i gruppen med høj familiær risiko. Desuden fandt vi en lav serotonintransporter forekomst i

hjerneområderne dosolateral præfrontal kortex og anteriore gyrus cinguli i gruppen med høj familiær risiko.

Disse resultater tyder på at ændringer i den serotonerge neurotransmission forekommer hos raske individer med høj risiko for at udvikle affektiv sygdom og således forudgår en eventuel senere sygdomsudvikling. Disse ændringer kan tænkes at opstå i løbet af den tidlige udvikling af hjernen og/eller som kompensation for lave niveauer af synaptisk serotonin, men det er forhold, vores studier ikke direkte kan belyse.

Vores fund kan inspirere fremtidige longitudinelle studier med formålet at identificere hvilke karakteristika ved den serotonerge neurotransmission, der spiller en rolle for senere udvikling af affektiv sygdom.

Contents

List of papers

1. Frokjaer VG, Mortensen EL, Nielsen FÅ, Haugbol S, Pinborg LH, Adams KH, Svarer C, Hasselbalch SG, Holm S, Paulson OB, Knudsen GM. Frontolimbic serotonin 2A receptor binding in healthy subjects is associated with personality risk factors for affective disorder (Biol Psychiatry 2008).

2. Frokjaer VG, Vinberg M, Baare W, Erritzoe D, Baare W, Holst KK, Mortensen EL, Arfan H, Madsen J, Kessing LV, Knudsen GM. Frontolimbic serotonin 2A receptor binding, neuroticism, and familial risk for mood disorder (submitted).

3. Frokjaer VG, Vinberg M, Erritzoe D, Svarer C, Baare W, Madsen K, Madsen J, Kessing LV, Knudsen GM. High familial risk for mood disorder is associated with low dorsolateral prefrontal cortex serotonin transporter binding (submitted).

Terminology and abbreviations

Affective disorder is used synonymously of *mood disorder*, in paper 1.

[^{11}C]DASB: ^{11}C-labeled 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile.

BDI-21: 21-item Beck Depression Inventory

BDI-14: 14-item Anxiety subscale

BMI: Body Mass Index

BP_{ND}: Binding potential, the ratio at equilibrium of specifically bound tracer to that of nondisplaceable tracer in tissue.

BP_{P}: Binding potential, the ratio at equilibrium of specifically bound tracer to that of total parent tracer in plasma.

DLPFC: dorsolateral prefrontal cortex

DZ: dizygotic

Endophenotype: any hereditary characteristic that is normally associated with a disorder but is not a direct symptom of that disorder.

[^{18}F]altanserin: ^{18}F-labeled 3-(2-[4-(4-fluorobenzoyl)-1-piperidinyl]-ethyl)-2,3-dihydro-2-thioxo-4-quinazolinone.

HAM-D: The Hamilton Depression Scale, 17-item

High-risk twins: mono- and dizygotic twins with a co-twin diagnosis of affective disorder.

5-HT: 5-hydroxytryptamine, serotonin

5-HT_{2A}: the serotonin 2A receptor

Low-risk twins: mono- and dizygotic twins with a mentally healthy co-twin and no first-degree relatives with affective or schizophrenic disorders.

Major depression and *depression* are used synonymously.

Mood disorders: include manic episodes, bipolar affective disorder, depressive episode and recurrent depressive disorder (ICD-10, F30-33).

MRI: Magnetic Resonance Imaging

MZ: monozygotic

Neuroticism: a personality component reflecting individual differences in the tendency to experience negative emotions such as cheerlessness, sadness, tension,

worries, and guilt. It is assessed from self-reported questionnaires; NEO-PI-R (Costa and McCrae 1992) or EPQ (Eysenck and Eysenck 1991).

PET: Positron Emission Tomography

SERT: serotonin transporter

SLE: Stress-full Life Events

SPECT: Single Photon Computed Tomography

Aims

The endeavor of the work presented here is to uncover possible relations between personality risk factors and familial risk of developing mood disorders and key elements in the serotonergic neurotransmission in the living human brain. Such studies can ideally support our understanding of why some individuals at high risk develop mood disorders and others do not. In a longer perspective, these studies may contribute to development of better treatment, early intervention, or maybe even preventive strategies for persons at high risk for developing mood disorders.

This thesis is based on 3 in vivo imaging studies that focus on the presynaptic serotonin 2A receptor and the postsynaptic serotonin transporter binding and distribution in relation to risk for developing mood disorder.

In study 1, we aimed at examining the possible association between the personality risk factor, neuroticism, and serotonin 2A receptor binding in regions of relevance to personality and mood disorders in a large sample of healthy volunteers.

In study 2, we aimed at testing the hypothesis generated in study 1, that the association between high neuroticism and high frontolimbic serotonin 2A receptor binding might be mediated by familial risk factors for developing mood disorders.

In study 3, we aimed at exploring whether the serotonin transporter binding in brain regions relevant to mood disorders might represent a trait characteristic of mood disorder.

Background and hypotheses

Mood disorders

Mood disorders include depressive episodes and recurrent depressive disorder, manic episodes, and bipolar disorder (ICD-10, F30-33) (WHO 2005). The predominant feature is disturbances in mood. The life time prevalence of unipolar depression, is at least 10%, being twice as common in women than in men (Hasin et al 2005). The life time prevalence of bipolar disorder, or manio-depressive disorder, is around 1-3% (Pini et al 2005). Depressive disorders are characterized by symptoms of depressed mood, irritability, low self-esteem, feelings of hopelessness and worthlessness, feelings of inappropriate guilt, sleep disturbances, altered appetite and weight, diminished interest or pleasure, and recurrent thoughts of death and suicide. Bipolar disorders are characterized by depressive episodes accompanied by manic episodes. Patients in a manic episode experience inflated self-esteem or grandiosity, decreased need for sleep, flight of ideas, pressure to talk, distractibility, and tend to behave irresponsibly and impulsively, and to involve themselves excessively in pleasurable activities that may have painful consequences later on.

It is not clear whether unipolar and bipolar disorders are categorically distinct or whether they lie on a continuous spectrum (Akiskal and Benazzi 2006). Typical age of onset for bipolar disorder is late adolescence or early adulthood (Pini et al 2005), whereas unipolar major depression is diagnosed slightly later, typically in early adulthood and progressively until the age of 40-45 years (Hasin et al 2005).

Time of debut, and the latency from debut to diagnosis and effective treatment, is crucial for the negative impact these disorders have on the lives of the patients and their relatives, since these periods of adolescent and adult life are often paramount in establishing adult social networks and family, education, and employment. The World Health Organization estimates that, worldwide, depression will become the second most important cause of disability or premature death by 2020 (Murray and Lopez 1996).

Genetic and environmental influence in the etiology and pathophysiology of mood disorders

The most potent risk factor for mood disorder is a family history of mood disorder, but the specific factors that are transmitted in families are not well elucidated. Mood disorders are elicited through a combination of genetic and environmental stress factors, and disturbances in serotonergic transmission seem to be an important pathophysiological component.

The heritability of depression, estimated from twin studies, is approximately 40% (Kendler et al 2006b; Kendler et al 1993a; McGuffin et al 1991; Sullivan et al 2000) higher in women than in men. Although modest single gene effects have been identified, the heritability is predominantly polygenic in nature (Levinson 2006). Recurrence and early age of onset characterize cases with the greatest familial risk (Kendler et al 1999; Kendler et al 1993a; Lyons et al 1998).

Genetic influence

Current studies of the genetic background for depression focus on two phenotypes, major depression and the personality trait neuroticism, a predictor of future onset of depression (Fanous et al 2007; Kendler et al 1993b). A genome-wide linkage study identified 3 chromosome regions containing genes possibly contributing to the risk of developing depression (Holmans et al 2007). Association studies of candidate genes have explored functional polymorphisms relevant to serotonergic neurotransmission. Small single gene effects of the short-allele polymorphism in the promoter region of the serotonin transporter gene have been demonstrated in bipolar disorder, suicidal behavior and depression-related personality trait neuroticism, but not directly in major depression, as reviewed by Levinson (2006). No single gene effects of polymorphisms of the serotonin 2A receptor gene have been identified in either neuroticism (Jonsson et al 2001; Kusumi et al 2002; Oswald et al 2003; Tochigi et al 2005), or depression (Jonsson et al 2001; Kusumi et al 2002; Oswald et al 2003; Tochigi et al 2005), again emphasizing the polygenic nature of the heritability characterizing both depression and neuroticism (Shifman et al 2007).

Gene by environment effects

Genetic vulnerability is modulated by certain environmental factors. In a seminal paper by Caspi et al (2003) it was established that the low expressing ”s” allele of the SERT promoter gene in combination with stressful life events greatly enhanced the risk of developing major depression. This work has been replicated by some (Kendler et al 2005) but not others (Zammit and Owen 2006). The observation that depression is twice as frequent in women may be due to gender-specific differences in genetic vulnerability to environmental stress (Barr et al 2004; Brummett et al 2007). Also “protective” or “opportunity” genes are likely to play a role in the context of mood disorders, presumably penetrating through interactions with beneficial environment factors. For example, Jokela et al. (2007) observed such an “opportunity gene” candidate or a genetically driven ability to benefit from positive environmental factors; subjects with the 102tc polymorphism of the serotonin 2A receptor gene, were responsive to the protective aspects of childhood maternal nurturance with regard to later development of depressive symptoms.

Environmental influence

Potent risk factors include stressful life events, cortisol hypersecretion (Bhagwagar et al 2003; Mannie et al 2007; Modell et al 1998; Portella et al 2005), and the personality trait neuroticism (Fanous et al 2007; Kendler et al 1993b). In particular, early exposure to stress such as maternal stress in fetal life, deprivation of normal parental care during infancy, or physical maltreatment possibly induces a long-lasting increased responsiveness to stress and is associated with increased risk of later development of depression (Heim and Nemeroff 2001; Kajantie 2006; Talge et al 2007)

Neuroprotective processes are also likely to be important in the pathophysiology of depression. One such neuroprotective molecule, brain derived neurotrophic factor (BDNF), is associated both with depression (Karege et al 2002) and with neuroticism (Lang et al 2004). However, BDNF has not been demonstrated to be a direct trait-characteristic of the proneness to develop depression, but especially in females, it seems to be a component of an adverse response to stress (Trajkovska et al 2007).

Yet, it is still poorly understood why some individuals at genetic and/or environmental risk develop mood disorders and others do not. This thesis focuses on

possible contributions from individual differences in serotonergic neurotransmission in brain regions of importance in mood disorders.

Brain regions of particular importance in mood disorders

Frontal cortex, hippocampus, anterior and posterior cingulate, as well as striatal brain regions are involved in major depression This has been confirmed by, e.g., brain morphological studies (Sheline 2003), and in functional (fMRI) studies where striatal regions are implicated in the disturbed processing of negative emotional stimuli (Anand et al 2005).

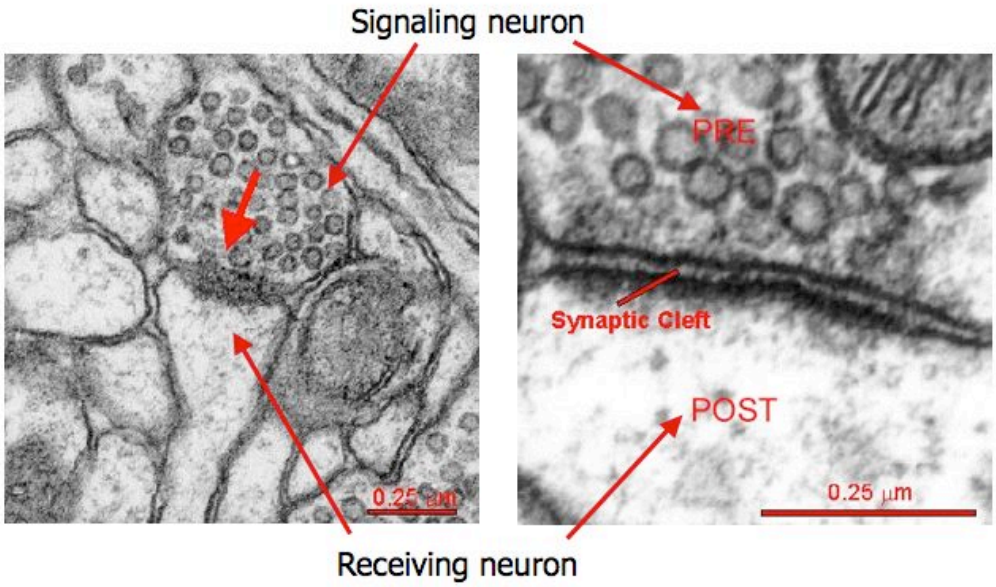
Within the frontal regions, particularly the dorsolateral prefrontal cortex (Brodmann 9 and 46) seems to play a key role in the pathophysiology of major depression. This has been documented by several different techniques, e.g., postmortem studies (Austin et al 2002; Mann et al 2000; Rajkowska et al 2001; Rajkowska et al 1999) and functional neuroimaging studies based on emotional and cognitive activation paradigms and studies of treatment effects, as reviewed by Fitzgerald et al (2006). Also, in the anterior cingulate metabolic (Drevets et al 1997; Mayberg et al 1997; Videbech 2000), structural (Caetano et al 2006; Sheline 2003) and functional changes (Neumeister et al 2004) has been linked to mood disorders. For an overview see Drevets et al (2000).

Prefrontal cortex, hippocampus, anterior cingulate are all part of, or intimately connected to, the “limbic system” that plays a key role in processing of emotions, mood regulation, and generation of stress and fear responses.

Serotonergic neurotransmission

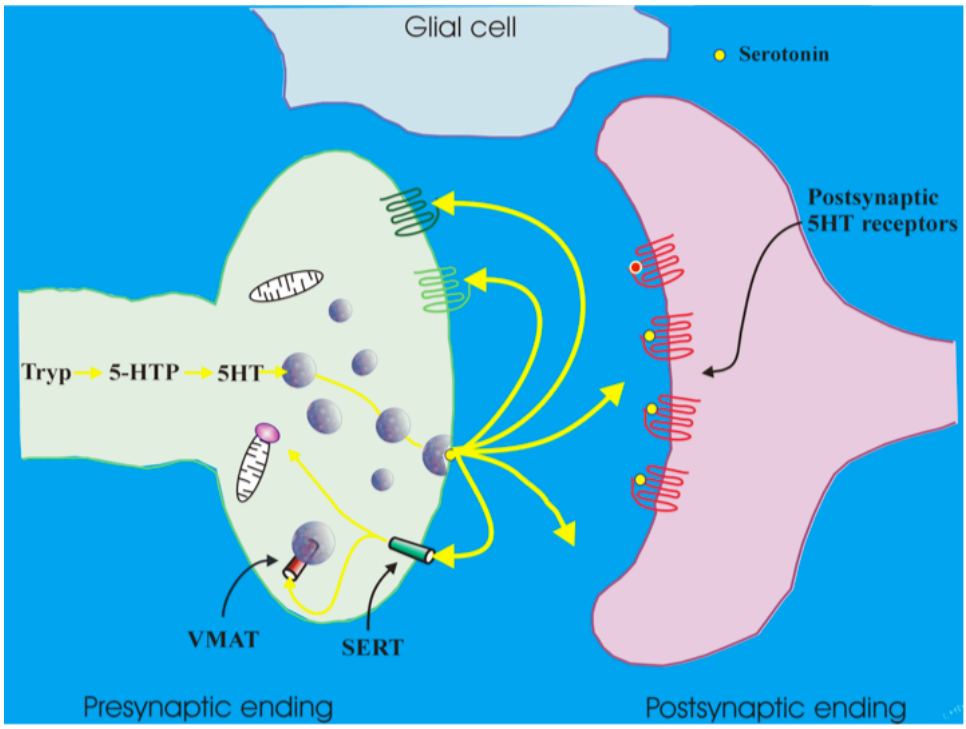
Serotonin (5-hydroxytryptamin) acts a transmitter in the central nervous system to modulate a wide spectrum of behaviors such as mood, anxiety, aggression, and psychophysiological functions such as appetite and sleep (Roth et al 1998). Coupled neurons communicate through chemical synapses, figure 1.

Figure 1. Electron microscopical image of a chemical synapse. Presumably an excitatory synapse in the hippocampus.



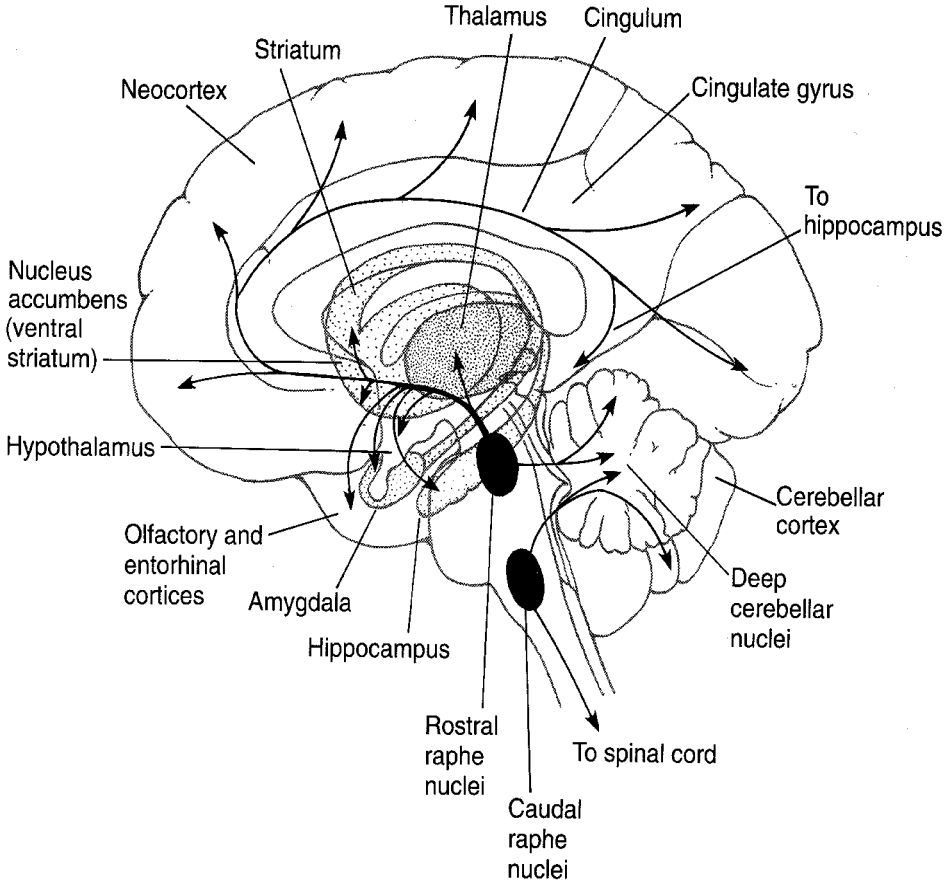
The effects of serotonin, released by serotonergic neurons, are mediated through multiple pre- and postsynaptically located receptors divided into 7 distinct serotonin receptor families. To date there are at least 15 known 5-HT receptor subtypes, whereas primarily one single protein, the serotonin transporter (SERT), is responsible for terminating the serotonergic signal. SERT is located presynaptically and via uptake of serotonin it clears the synapse, recycles the transmitter, terminates the signal and leaves the system ready for a new signal, as illustrated in figure 2. SERT is located on the cell bodies and terminals of serotonergic neurons and represent a marker of serotonergic innervation (Blakely et al 1994). The serotonin 1A presynaptic autoreceptor plays a key role in the regulation of serotonergic transmission. Stimulation of the presynaptic serotonin 1A autoreceptor in raphe nuclei inhibits serotonin release (Barnes and Sharp 1999).

Figure 2. Serotonergic synapse.



Serotonergic neurons originate at brain stem level from the raphe nuclei and project to limbic and cortical structures, practically, within the entire brain as illustrated in figure 3. The regional location of different postsynaptic receptor subtypes is highly specific. The serotonin 2A receptor is primarily distributed in the cerebral cortex whereas intermediate binding is observed in insula and in subcortical regions such as thalamus and nucleus caudatus (Adams et al 2004; Varnas et al 2004). SERT is located with high density subcortically; in midbrain, thalamus, putamen, caudatus, and in intermediate binding is seen in the anterior cingulate and frontal cortex (Kish et al 2005; Varnas et al 2004). Cerebellum, except for the vermis part, has only negligible amounts of serotonin 2A receptors and SERT (Cortes et al 1988; Kish et al 2005; Pazos et al 1987).

Figure 3. Projections from serotonergic neurons.



Serotonergic neurotransmission - involvement in mood disorders

Several antidepressants act on the serotonergic neurotransmission. SERT is the targeted site of action of SSRIs (selective serotonin reuptake inhibitors). The subsequent rise in synaptic serotonin and modulation of serotonergic neurotransmission that include down-regulation of SERT and serotonin 2A receptors are believed to be important for the antidepressant effects (Benmansour et al 2002; Gray and Roth 2001). Also, direct serotonin 2A receptor inhibition mediate antidepressant effects, and genetic variance in the gene encoding the serotonin 2A receptor is associated with the response to treatment with citalopram (McMahon et al 2006).

Other pre- and postsynaptic receptor sites are also implicated in the pathophysiology of mood disorder and the mediation of treatment effects, e. g. serotonin 1A presynaptic autoreceptors appear to be associated with the effects of SSRIs. The clinical effect of SSRIs is usually not observed until several weeks after initiation of treatment. This delay may be due to the time it takes for the serotonin 1A

autoreceptors in the raphe nuclei to desensitize (Blier et al 1990; Celada et al 2004). The serotonin 1A receptor is also altered in patients with mood disorder off medication. Most often a decrease in cortical serotonin 1A receptor binding has been reported as reviewed by Drevets et al (2007). However, also increased serotonin 1A receptor binding has been reported in antidepressant-naive depressed patients in both raphe and cortical regions (Parsey et al 2006b) and, postmortem, in raphe nuclei (Stockmeier et al 1998). The latter suggesting an over expression of inhibitory presynaptic serotonin 1A receptors in depression. Recently, serotonin 4 receptor agonists have been pointed out as a promising target for antidepressant treatment with rapid onset (Lucas et al 2007). The role of the many other receptor sites involved in serotonergic neurotransmission cannot yet be evaluated in vivo due to the lack of suitable radioligands.

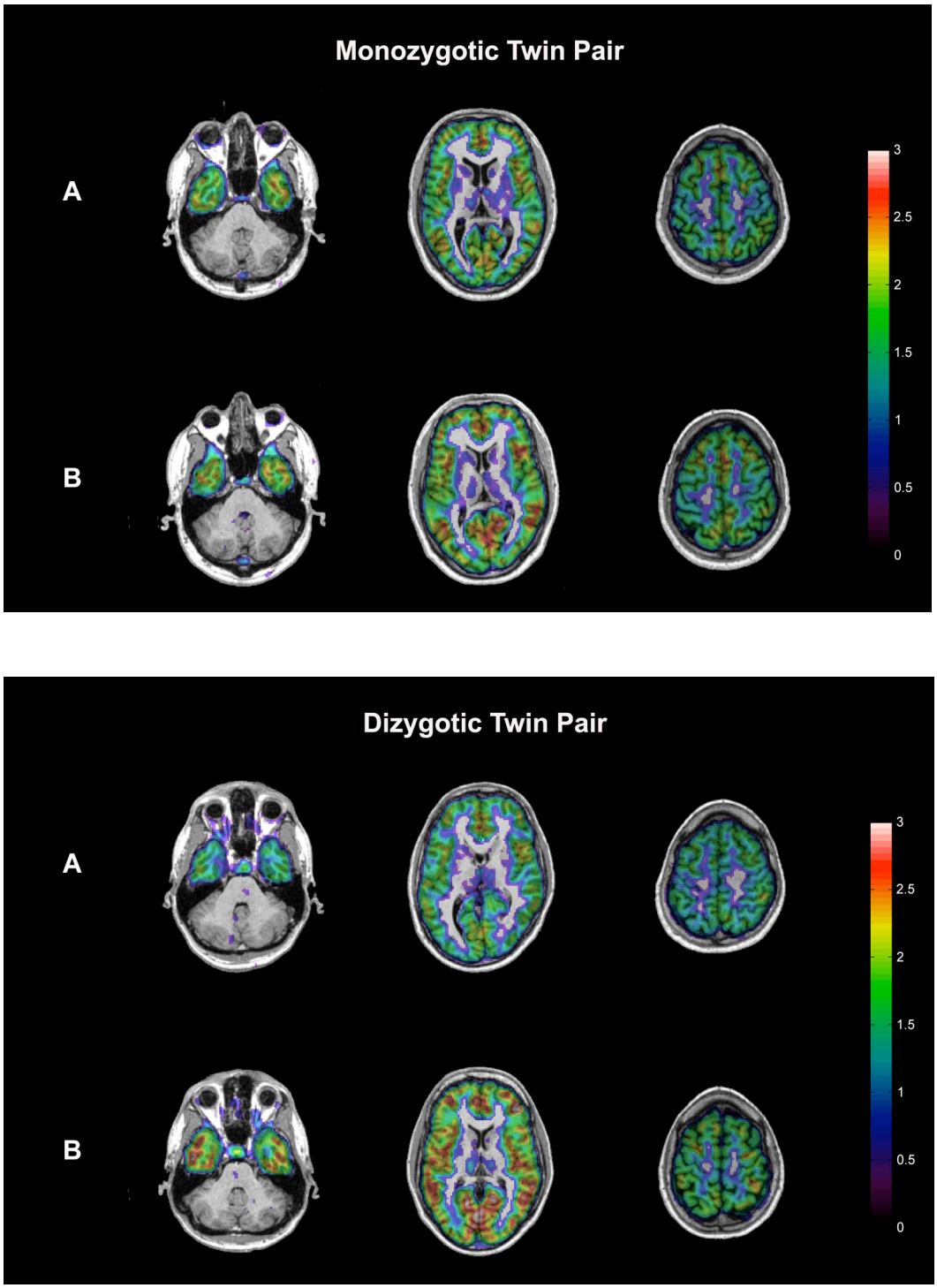
In this thesis we focus on the postsynaptic serotonin 2A receptor and the presynaptic SERT and study whether they represent neurobiological correlates of personality and familial risk factors for mood disorders.

Can elements of serotonergic neurotransmission represent a heritable risk factor?

A fundamental premise of identifying a neurobiological characteristic, e.g. the serotonin receptor levels and distribution, as a heritable risk factor for mood disorders is that genetic mechanisms can account for differences in the system studied. Pinborg et al (2007) has provided such evidence with regard to the serotonin 2A receptor by showing that the serotonin 2A receptor setting and pattern of distribution is tighter correlated in monozygotic twins than in dizygotic twins and therefore is indeed heritable, see figure 4. Also, individual specific environmental factors contributed only modestly to the variance, indicating that after early life the serotonin 2A receptor pattern remains fairly constant (Pinborg et al 2007).

Nevertheless, the serotonin 2A receptor and SERT do respond to environmental factors, e.g. stress and high plasma cortisol (Dwivedi et al 2005; Tafet et al 2001) and serotonin 2A is also modulated by postnatal levels of low brain derived neurotrophic factor (Rios et al 2006). Therefore, early environmental factors as well as adult life environment may also influence serotonergic neurotransmission critically.

Figure 4. Serotonin 2A receptor binding in a monozygotic and a dizygotic twin pair (from Pinborg et al. 2007).



Challenge studies in relatives to depressive patients and in recovered, un-medicated euthymic patients suggest that some aspects of the impaired serotonin neurotransmission are trait characteristics of vulnerability to depression. Individuals with a family history of affective disorder are more prone to develop depressive symptoms when depleted of brain tryptophan, the precursor of serotonin (Klaassen et al 1999; Quintin et al 2001). Also, Bhagwagar et al (2002) found that currently depressed patients as well as recovered patients have a decreased functional hypothalamic response to serotonin challenge. These studies suggest a genetic vulnerability to serotonergic dysfunction associated with familial risk of mood disorders.

Serotonin 2A receptor binding in mood disorders

The role of the serotonin 2A receptor in depression has been extensively studied; the majority of post mortem studies in suicide victims of major depression report increased serotonin 2A receptor binding in dorsolateral prefrontal cortex (Arango et al 1997; Stockmeier 2003). Table 1 summarizes main findings of brain imaging studies of serotonin 2A in mood disorder. Although initial findings of in vivo receptor imaging studies were contradictory two recent studies have confirmed the postmortem data in recovered, un-medicated patients with a history of major depression (Bhagwagar et al 2006), and in un-medicated patients with severe depression (Meyer et al 2003), as discussed in further detail in paper 1 and 2. To my knowledge no imaging studies of the serotonin 2A receptor have been conducted in patients with bipolar disorder.

Accordingly, postmortem data and recent in vivo imaging data, based on highly specific tracers and patients that were not recently treated with antidepressants, are converging to support that a high serotonin 2A receptor binding is implicated in depression. However, it is not clear whether this represents a trait or a state marker of depression.

Table 1. In vivo imaging studies of serotonin 2A receptors in depression.

Tracer	Citation/year	Diagnosis	N	Medication	Primary findings
[123I]ketanserin	D'Haenen et al 1992	Depression	19(10)	Medication free 7 days	Parietal cortex↑ Left, relative to right prefrontal cortex↑
[18F]setoperone	Attar-Levy et al 1999	Depressive episode	7(7)	Benzodiazepines	Prefrontal cortex↓
	Meyer et al 1999	Current depressive episode, secondary to MDD	14(19)	Medication free (>6 months)	Prefrontal cortex→
	Yatham et al 2000	MDD	20(20)	Medication free (2 weeks)	Cortex↓
	Messa et al 2003	Current depressive episode	19(20)	Antidepressant naive Benzodiazepines	Cortex↓
	Meyer et al 2003	Current depressive episode, secondary to MDD	22(22)	Medication free (6 months)	Cortex↑ (particularly dorsolateral prefrontal) in severe depression Positive association with dysfunctional attitudes
[18F]altanserin	Biver et al 1997	MDD	8(22)	Medication free (10 days)	Orbitofrontal cortex↓
	Meltzer et al 1999	Late-life depression (mean age: 65)	11(11)	Untreated	Cortex→
	Mintun et al 2004	Current depressive episode	46(29)	Medication free (4 wk)	Hippocampus↓ Cortex→
[11C]MDL	Bhagwagar et al 2006	Prior, recurrent depression ≥2 episodes	20/20	Medication free > 6 months	Frontal, parietal, occipital↑ Positive correlation with dysfunctional attitudes in recovered patients

N: number of participating patients with healthy controls in brackets. MDD: major depressive disorder.

Serotonin transporter (SERT) binding in mood disorders

As reviewed by Stockmeier (2003), most postmortem studies in depressed and/or suicide subjects show either a decreased or no change in prefrontal SERT binding, and no changes in the brainstem. Only two postmortem studies report from the brainstem (Arango et al 2001; Klimek et al 2003), both reporting no change in the serotonin receptor density in postmortem tissue from depressive subjects. Arango et al (2001) suggest a decrease in dorsal raphe volume, but no decrease in serotonin transporter density. As summarized in table 2, in vivo imaging studies of the SERT in mood disorders show mixed results in *subcortical* regions observing either decreased (Malison et al 1998; Oquendo et al 2007; Parsey et al 2006a; Willeit et al 2000), no changes (Meyer et al 2004; Meyer et al 2001), or increased SERT binding (Cannon et al 2006; Cannon et al 2007; Dahlstrom et al 2000; Ichimiya et al 2002; Meyer et al 2004). In vivo imaging of *cortical* SERT show unchanged or elevated SERT-binding levels (Cannon et al 2006; Meyer et al 2004; Meyer et al 2001) as discussed in further detail in paper 3.

Genetic mechanisms affecting the SERT-expression, e.g. the low expressing "s" allele (Caspi et al 2003), are potential important mediators of vulnerability to depression as mentioned above. Whether this is due to a direct effect on in vivo SERT availability at any time during life is not yet clear (van Dyck et al 2004; Willeit et al 2001). However, recent studies indicate that functional subtypes of the high expresssing "l" allele do translate into differences in vivo SERT-binding in putamen (Praschak-Rieder et al 2007) or thalamus (Reimold et al 2007). Serotonergic modulations early in life, in particular low levels of SERT through early brain development (Ansorge et al 2004; Lira et al 2003), may also play a role since serotonin is critical in the maturation of brain systems that modulate emotional function in the adult (Gaspar et al 2003).

Table 2. In vivo imaging studies of serotonin transporters in mood disorder.

Tracer	Citation/year	Diagnosis	N	Medication	Primary findings
[123I]β-CIT	Malison et al 1998	Major depression	15(15)	6 drug-naive, 9 medication free for 3 wk	Midbrain/brainstem↓
	Willeit et al 2000	Seasonal affective disorder,	11(11)	6 drug naive, medication free >6 mo	Thalamus/hypothalamus↓ Midbrain/brainstem→
	Dahlstrom et al 2000	Depression, children/ adoloscents,	41(8)	Drug-naive	Thalamus/hypothalamus↑
[11C]McN5652	Ichimiya et al 2002	Uni and bipolar depression	11(11)	Medication free for 6 wk	Thalamus↑
	Parsey et al 2006	Major depressive episode	25(43)	12 antidepressant naive, antidepressant free >2 mo, no control for benzodiazepines	Amygdala, midbrain↓
	Oquendo et al 2007	Depressed bipolar disorder	18(41)	Off habitual antidepressants for >2wk, (fluoxetine >6 wk, neuoleptics >3wk, benzodiazepines >24 hr)	Thalamus, hippocampus, amygdala, putamen, anterior cingulate, midbrain↓
[11C]DASB	Meyer et al 2001	Major depression	12(17)	Antidepressant free > 2 mo	Striatum →
	Meyer et al 2004	Depressive episodes, DSM-IV	20(20)	14 drug naive, medication free for >3 mo	Prefrontal cortex, anterior cingulate, thalamus, caudatus, putamen → ↑ in severe depression
	Cannon et al 2006	Depressed, bipolar disorder	18(37)	Drug-free > 3wk, (fluoxetine >8 wk)	Thalamus, dorsal cingulate, medial prefrontal cortex, insula↑ brainstem↓
	Cannon et al 2007	Major depression and bipolar disorder	18 18 (34)	Drug-free > 3wk, (fluoxetine free >8 wk)	Thalamus, insula, striatum↑, MDD: brainstem↑ BD: brainstem↓
	*Bhagwagar et al 2007	Prior, recurrent depression ≥2 episodes	24 (20) all males	9 antidepressant naive. 15 drug-free > 3 mo	Amygdala, anterior cingulate, caudate, frontal cortex, hippocampus, insula, dorsal raphe, thalamus→
	*Reimold et al 2008	Unipolar depression	10 (19)	3 antidepressant naive, 7 antide-pressant withdra-wal for 5-17 days, 2 on neuroleptics	Thalamus ↓ Midbrain, amygdala → Negative association with state anxiety overall

N: number of participating patients with healthy controls in brackets. MDD: major depressive disorder.

BD: bipolar disorder. *These studies were added after the thesis was handed in January 2th 2008.

Hypotheses

1. Neuroticism is correlated to serotonin 2A receptor binding in healthy individuals in brain regions of relevance to personality and mood disorders.

Based on the outcome from study 1 we hypothesized that:

2. The coupling between a high frontolimbic serotonin 2A receptor binding and high neuroticism is more prominent in individuals at high than low familial risk of developing a mood disorder.

3. The serotonin transporter binding is as a trait marker for future development of mood disorders. Accordingly, altered serotonin transporter binding in frontal and subcortical regions of relevance in depression will characterize individuals at high familial risk as compared to individuals at very low risk.

Methods

Strategies for testing trait characteristics or risk factors for mood disorders

We applied two different strategies for studying potential trait characteristics of mood disorders with respect to serotonergic neurotransmission.

1. Linking personality risk factors for mood disorder to serotonin 2A receptor binding.

Since neuroticism is a robust predictor of future development of depressive episodes (Fanous et al 2007; Kendler et al 1993b), and neuroticism share considerable genetic overlap with depression (Kendler et al 2006a) it may constitute an "endophenotype" of risk for mood disorder. Therefore studying neurobiological characteristics associated with neuroticism will allow characterization of brain biology relevant for mood disorders. The advantage of using endophenotypes is that it allows avoidance of confounding effect of current or prior depression, of antidepressant treatment, and it can be more clearly defined than the heterogenous spectrum of mood disorders.

2. Linking of inherited risk through family history of mood disorder to serotonin 2A receptor or SERT binding.

We studied individuals at high and low familial risk of developing mood disorders. Healthy twins with a co-twin history of mood disorder (high-risk) and without a first degree relative history of mood disorders (low-risk) were identified by linking information from the Danish Twin Register and the Danish Psychiatric Central Register (described below). Studies of twins are particularly valuable in mood disorders, since twins share both early environmental factors and also a large fraction of their genes. A study of healthy twins at high risk versus low risk would yield maximal contrast and would also avoid confounding effects of prior or current psychiatric disorder, including effects of antidepressant treatment. It should be emphasized that the study was not designed as a traditional twin study, to answer questions about the relative influence of genetic versus early environmental factors. Rather, our aim was to identify healthy subjects at high or low risk for mood

disorders, and the twins (even the DZ) served that purpose better than other relatives to patients with mood disorders.

Personality measurements

Personality was characterized by the self-reported questionnaire NEO-PI-R (NEO Personality Inventory Revised), 240 item NEO-PI-R Danish version (Skovdahl-Hansen 2004). NEO-PI-R evaluates the broad personality dimensions of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness. Each dimension or factor score is derived by adding the scores from assessment of six personality traits (facets) and each trait score is derived by adding the scores on 8 items in 0 – 4 Likert format (Costa and McCrae 1992), see examples of items in table 3. The Danish translation of the NEO-PI-R has been psychometrically evaluated and normed in a standardization sample of 600 subjects. The constituent traits of neuroticism are: anxiety, depression, self-conscientiousness, vulnerability, impulsiveness, and angry hostility. Five of these traits included in the neuroticism score have high factor loadings on the neuroticism dimension (0.67-0.84) while the loading for impulsiveness is lower (0.37) (Skovdahl-Hansen 2004). This is in line with the American version of NEO-PI-R (Costa and McCrae 1992). NEO-PI-R has been shown to be stable across cultures (Terracciano et al 2005) and stable throughout adult life (Caspi et al 2005).

The personality dimension neuroticism as assessed from NEO-PI-R (Costa and McCrae 1992) or the EPQ (Eysenck and Eysenck 1991) reflects individual differences in the tendency to experience negative emotions such as cheerlessness, sadness, tension, worries, and guilt. Personality traits show considerable heritability. For the neuroticism dimension of the NEO-PI-R the heritability is around 40% (Jang et al 1996). In a large twin population of 20.692 members of same-sex twin pairs the genetic correlation between neuroticism and depression was estimated to be +0.46 (Kendler et al 2006a). This correlation is not clearly gender specific (Fanous et al 2002).

As reported in paper 1, individuals volunteering for a PET scan display slightly higher openness scores, whereas the remaining dimensions of NEO-PI-R, including neuroticism, are not different from that of the general population (Skovdahl-Hansen 2004).

Table 3. Examples of NEO-PI-R statements for assessing vulnerability, anxiety and depression components of neuroticism.

"I can handle myself pretty well in a crisis"
"I often feel helpless and want someone else to solve my problems"
"It's often hard for me to make up my mind"
"I'm seldom apprehensive about the future"
"I rarely feel lonely or blue"
"I tend to blame myself when anything goes wrong"
"I often worry about things that might go wrong"

Registers used in the high-risk low-risk study design

The Danish Civil Registration System assigns a unique personal identification number to all residents in Denmark. This number is linked to information on name, address, and date of birth; and information on death, emigration and immigration is also recorded in the system. All other Danish registers use the same unique identifier and thus Danish residents can, for research purposes, be tracked in all the public registers through record linkage.

The Danish Psychiatric Central Research Register is a nationwide registration of all psychiatric admissions and outpatient hospital contacts in Denmark for the country's 5.3 million inhabitants (Munk-Jorgensen and Mortensen 1997). From April 1969 to December 1993, diseases were classified according to the International Classification of Diseases,"8th"(ICD-8) (WHO 1967) and from January 1994 according to the International Classification of Diseases, "10th" (ICD-10) (WHO 2005). "Affective disorder" in ICD-10 is used synonymously with "mood disorder" in DSM-IV.

The Danish Twin Registry was initiated in 1953. By 2006 it contained information on 75.000 twin pairs born between 1870 and 2003. The completeness varies with the birth cohort and is approximately 70% for the period before the Civil Registration System was established (in 1968) and close to 100% after (Harvald et al 2004; Kyvik et al 1996).

The linkage identified same-sex twin pairs in which one twin had been treated in a psychiatric hospital setting for an affective episode and the other twin had not been treated for affective disorder, the high-risk healthy co-twin. Affected twins were identified as twins who on their first admission, in the period between 1968 and 2005, were discharged from a psychiatric hospital with a diagnosis of depression or recurrent depression (ICD-8-codes 296.09, 296.29, 296.89, 296.99; ICD-10-codes: F32-33.9) or a first diagnosis of manic mixed episode or bipolar affective disorder (ICD-8-Codes 296.19, 296.39; ICD-10-codes: F30-31.6, F34.0 F38.00). Twins at low risk of developing affective disorders were ascertained from twin pairs where none of the co-twins had a personal history of affective disorder, and matched on age, sex and zygosity for each twin at high risk.

Psychiatric screening and neuropsychiatric assessments are accounted for in detail in the method sections of paper 2 and 3. Follow-up has not yet been performed.

Measuring serotonergic neurotransmission in vivo in humans by PET

Positron Emission Tomography (PET) can measure receptor binding in the living human brain given that a suitable radiolabeled tracer is available for the receptor at interest. General tracer requirements include: the radiolabeled tracer should pass the blood brain barrier in sufficient amounts, a favourable ratio between tracer uptake in specific versus non-specific binding tissue, fast kinetics allowing quantification by kinetic modeling or fast achievement of steady state between tracer in plasma and in brain tissue, tolerable radioactivity dosages (ideally below 5 mSv), tolerable scanning paradigm (short acquisition time e.g. <90 min). Also, preferably, a suitable reference region should be present providing a measure for the non-specific binding of the tracer. The use of a reference region enables quantification without the need for arterial input measurements (Lammertsma and Hume 1996). Arterial input measurement is not only uncomfortable for the person being scanned, but also often adds noise to the receptor estimates due to methodologic challenges in measuring the plasma concentration of parent compound.

The following section briefly describes and discusses the methods we applied to image and quantify the serotonin 2A receptor and SERT binding. The methods are also described in more detail in the papers.

Serotonin 2A receptor imaging with [^{18}F]altanserin PET

^{18}F-labeled (3-(2-[4-(4-fluorobenzoyl)-1-piperidinyl]-ethyl)-2,3-dihydro-2-thioxo-4-quinazolinone), ([^{18}F]altanserin) is a 4-fluorobenzoyl-piperidine derivative, and a selective serotonin 2A antagonist. [^{18}F]altanserin binds with high affinity and high selectivity to the serotonin 2A receptor (Kristiansen et al 2005). In humans lipophilic metabolites of [^{18}F]altanserin, that do not bind specifically to serotonin 2 receptors, are produced and has to be incorporated in the quantification to avoid bias. In our experimental set-up we determine the binding potential BP_P with the bolus-infusion approach developed by Pinborg et al (2003). This approach is based on the presence of a tracer steady state in both brain tissue and plasma. The advantage over e.g. Logan analysis of bolus data (Price et al 2001) is that the contribution from radiolabelled lipophilic metabolites, if not representing specific binding, can be subtracted directly, provided that a suitable reference region void of receptors exists. Cerebellum is considered to contain negligible levels of serotonin 2A receptors (Pazos et al 1987) and can be used as a reference region even though age-related increase of cerebellar binding is observed (Adams et al 2004).

Metabolite measurements, acquisition procedures, reconstruction, and quantifi-cation are described in the method section of paper 1 and 2 and by Pinborg et al (2003).

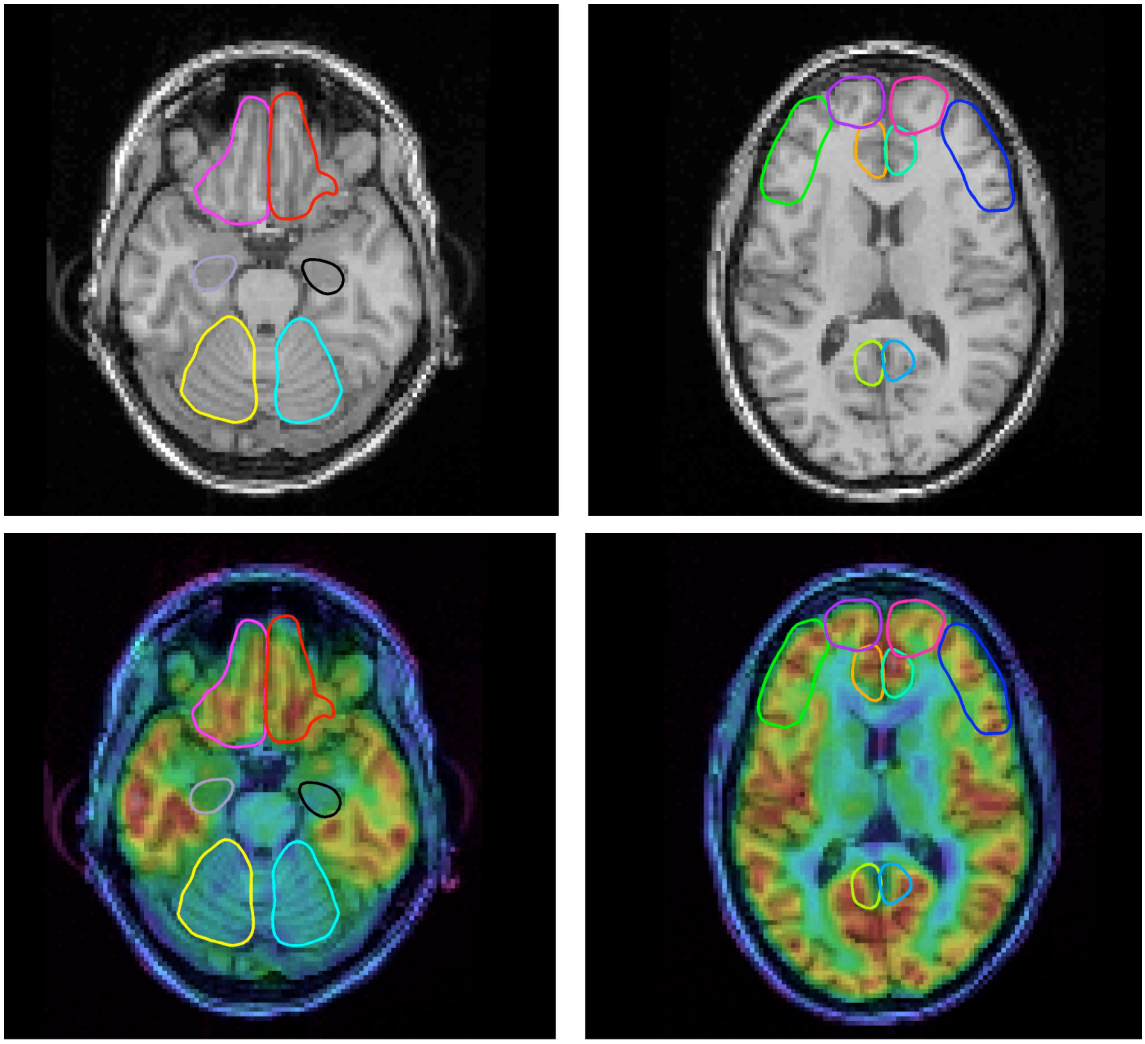
The outcome parameter is defined as follows:

$$BP_P = (C_{VOI} - C_{Reference}) / C_{Plasma} = f_p * (B_{max}/K_d) \text{ (mL/mL)}$$

where C_{ROI} and $C_{Reference}$ are mean counts in the volume of interest and in the reference region, respectively, C_{Plasma} is the radioactivity originating from parent compound in plasma, f_P is the free fraction of radiotracer, B_{max} is the density of receptor sites available for tracer binding, and K_d is the affinity constant of the radiotracer to the receptor.

The test-retest variability of [^{18}F]altanserin PET in our lab been determined as 5-12% in high-density cortical regions and 11-39% in low-density subcortical regions. Consequently, in most study designs [^{18}F]altanserin PET is primarily suitable for measuring cortical serotonin 2A receptor binding (Haugbol et al 2007).

Figure 5. [^{18}F]altanserin PET image and frontolimbic volumes of interest including: orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, anterior and posterior cingulate, and hippocampus. Upper panel: VOIs delineated on MRI. Lower panel: [^{18}F]altanserin PET and VOIs on MRI. Cerebellum is also delineated.

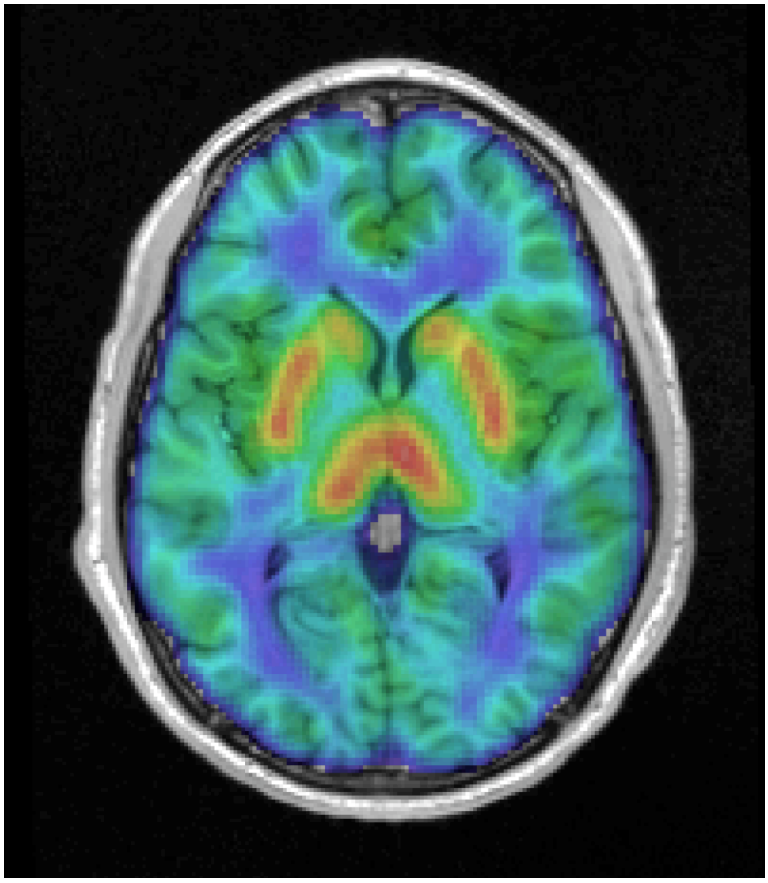


SERT imaging with [^{11}C]DASB PET

Recently, highly specific tracers were developed for imaging of the SERT binding. Imaging with ^{11}C-labeled 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile ([^{11}C]DASB) has become the favorite PET radiotracer due to its high specificity, fast kinetics, and favourable signal to noise ratio (Huang et al 2002; Meyer 2007). A SPECT (single photon emission computed tomography) tracer, [^{123}I]ADAM, is also available and has recently been validated in humans (Frokjaer et al 2008). The advantage of PET over SPECT is higher resolution and the larger number of short-lived isotopes available. Thus determination of regional binding pattern will be more precise with PET. In the study reported here SERT binding is imaged with [^{11}C]DASB PET as illustrated in figure 6. Our measurements are based on 90 minutes dynamic acquisition starting immediately after bolus injection, according to Ichise et al (2003). In this set-up regional SERT binding is quantified by estimating the binding potential (BP_{ND}) of specific tracer binding using simplified kinetic modeling methods (MRTM2) with cerebellum as a reference region and fixing of the clearance rate constant from cerebellum (k_2') as estimated from a high binding region including thalamus, putamen and caudate.

The test-retest variability of SERT BP_{ND} when measured according to Ichise et al (2003), as in paper 3, is 9% in frontal cortex, 9-13% in the remaining cortical regions, and 4-5% in subcortical regions (Kim et al 2006).

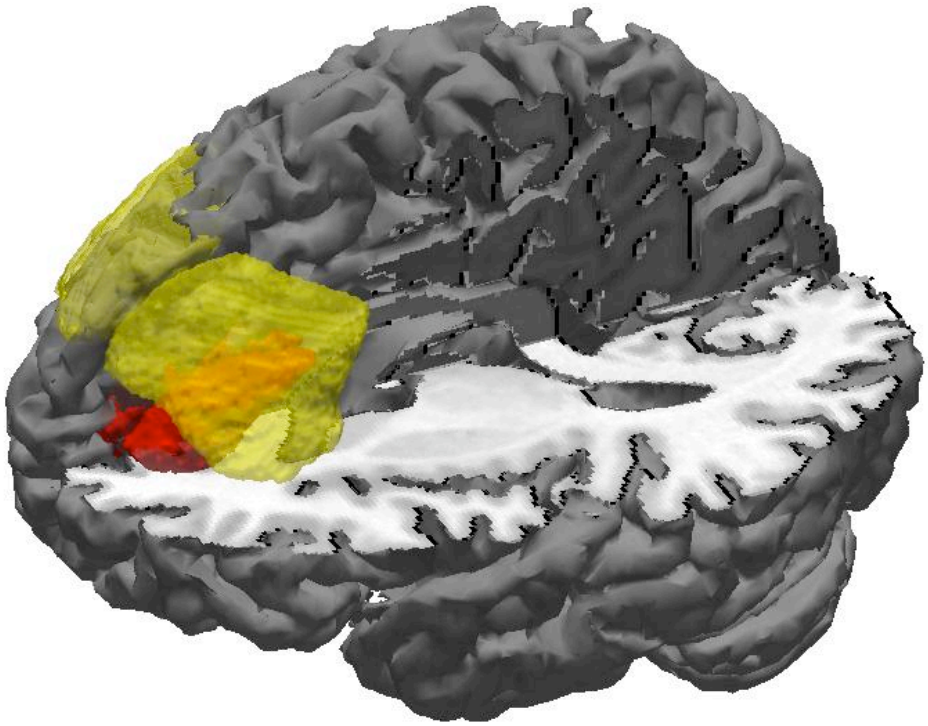
Figure 6. [^{11}C]DASB PET image projected onto a corresponding MRI.



Structural brain imaging with magnetic resonance (MRI) is conducted as a supplement to all PET-studies to allow for delineation of volumes of interest and partial volume correction based on segmentations of cerebrospinal fluid, white- and gray matter. Partial volume correction uses the anatomical information from the MRI to correct for the spill-in and spill-out from neighboring tissue in the smoothed PET image. Consequently, it is a correction for the low resolution in PET. In each of the studies reported here, all PET and MR images were processed, by the same person, and volumes of interest were delineated in a strictly user-independent fashion as described by Svarer et al. (2005). In figure 5 and 7 the volumes of interest are illustrated. Details about MR acquisition, bias correction, and segmentation of the MRIs are described in the papers. In paper 1 we abstained from partial volume correction since the cohort was scanned in two different MR-scanners, a 1.5 Tesla and a 3 Tesla scanner. Accordingly, the segmentation might vary significantly and this would possibly bias the findings. In paper 3 we included regions were the tissue structure does not allow for segmentation (midbrain and raphe). Therefore, partial

volume correction ad modum Müller-Gartner was not applied to allow comparability across regions. However, time-activity curves were extracted from gray matter volumes only, so atrophy was to some extent taken into consideration.

Figure 7. 3D volumes of interest projected onto MRI. Yellow: Dorsolateral prefrontal cortex. Red: Anterior cingulate (see study 3).



Age and BMI corrections

Both the serotonin 2A receptor (Adams et al 2004) and in several reports also SERT binding (Meyer et al 2001; Pirker et al 2000; van Dyck et al 2000) show an age dependent decrease in healthy volunteers, and, as reported, age adjustment was also appropriate in our studies. The serotonin 2A receptor binding was also adjusted for body mass index (BMI) in study 2 where it had a significant effect when added as a covariate. Nevertheless, in study 1, BMI did not contribute to the explanatory effect in the model, and we did not yet have an independent replication of the positive correlation between BMI and serotonin 2A receptor binding. Hence it was left out.

Results

The results are presented in detail in the papers covering study 1, 2, and 3. Here the findings are summarized.

Study 1: Frontolimbic serotonin 2A receptor binding in healthy subjects is associated with personality risk factors for affective disorder

This study show that the personality risk factor of depression, neuroticism, is positively associated with frontolimbic serotonin 2A receptor binding in a large sample of 83 healthy volunteers without self-reported first degree relatives with psychiatric disorders. This association was particularly strong for the constituent trait of neuroticism, vulnerability, defined as a person's difficulties in coping with stress. However, also the anxiety, self-conscientiousness, and depression component of neuroticism contributed.

These findings point out a link between personality risk factors for mood disorders and serotonergic neurotransmission. This link may be related to genetic risk factors of mood disorders.

Figure 8. The correlation between neuroticism and age-adjusted frontolimbic serotonin 2A receptor (5-HT_{2A}) binding.

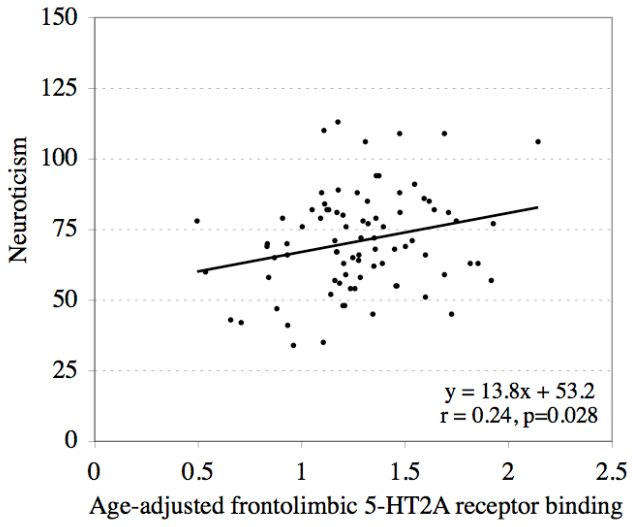
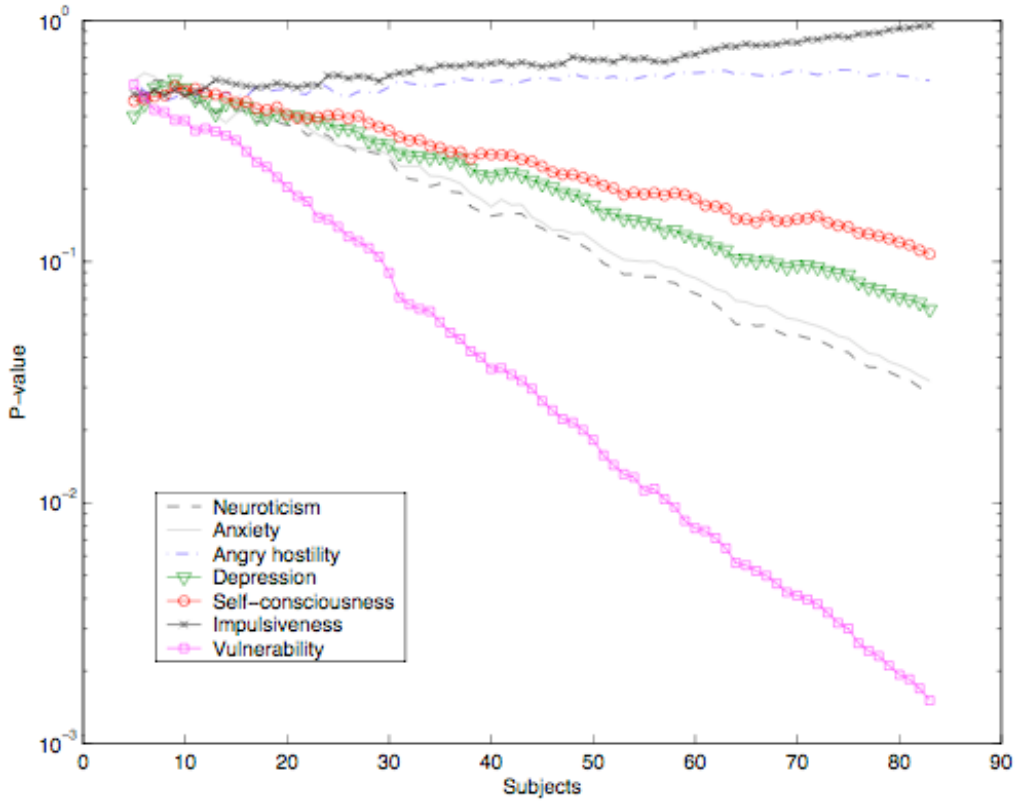


Figure 9. The correlations between the 6 constituent facets of neuroticism and frontolimbic serotonin 2A receptor binding as compared to the correlation with neuroticism itself as a function of number of included subjects. The calculated p-value for the positive correlation between each facet score and the frontolimbic serotonin 2A receptor binding for each number of subjects included (N: 1 to 83) is based on random resampling 100 times in the total cohort.



Study 2: Frontolimbic serotonin 2A receptor binding, neuroticism, and familial risk for mood disorders

This study shows that the coupling between frontolimbic serotonin 2A receptor binding and neuroticism is stronger in healthy twins at high familial risk for mood disorder than in healthy twins at low familial risk. Within the high-risk group (N=21), neuroticism and frontolimbic serotonin 2A receptor binding was positively associated in an analysis adjusting for age and BMI (p=0.0049), whereas this was not the case in the low-risk group (N=17) (p=0.25). This interaction between neuroticism and risk-status was significant (p=0.030). Further, in support of the hypothesis we saw that, on a trend basis, the groups of increasing risk-load (low-risk monozygotic/dizygotic <

high-risk dizygotic < high-risk monozygotic) showed the expected hierarchy in terms of increasing association between frontolimbic serotonin 2A and neuroticism.

These findings suggest that familial risk mediates an association between high frontolimbic serotonin 2A receptor binding and neuroticism.

Figure 10. Effect of risk status on the association between frontolimbic 2A receptor BP_P and neuroticism, adjusted for BMI and age. In the high-end of the neuroticism scale, the high-risk group showed an elevated BP_P, whereas, in the low-end they showed a decreased BP_P as compared to the low-risk group. Point-wise 95% symmetric confidence bands of the regression lines are displayed. The regression lines represent the associations given a mean BMI and mean age.

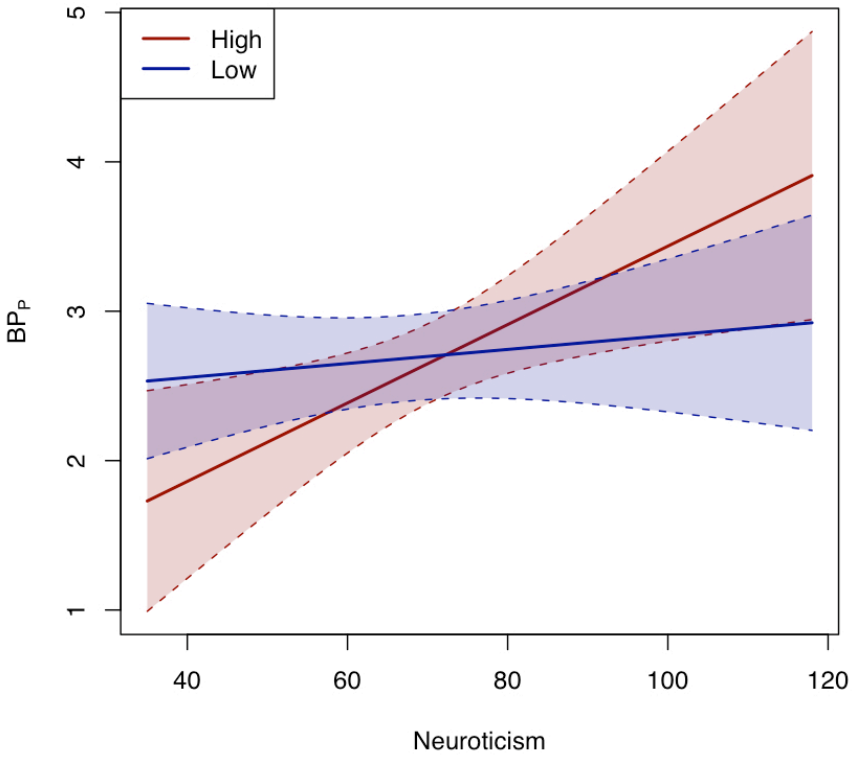
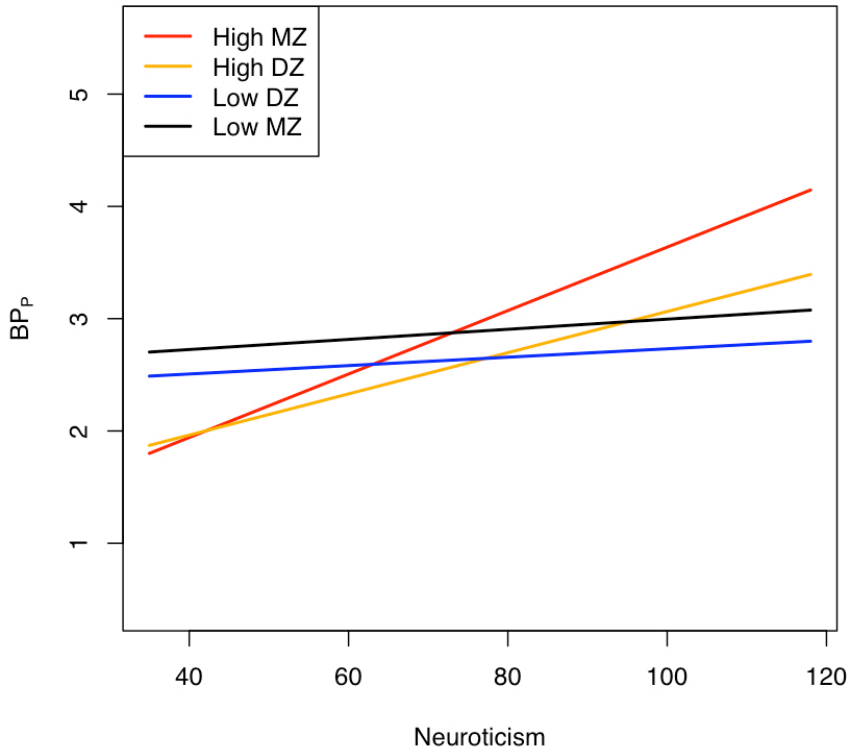


Figure 11. Effect of zygosity and risk on the association between frontolimbic serotonin 2A receptor binding and neuroticism, adjusting for BMI and age. Genetic risk-load: low-risk MZ/DZ < high-risk DZ < high-risk MZ. The high-risk-MZ subgroup shows the strongest association, followed by the high-risk-DZ group, and the low-risk group.

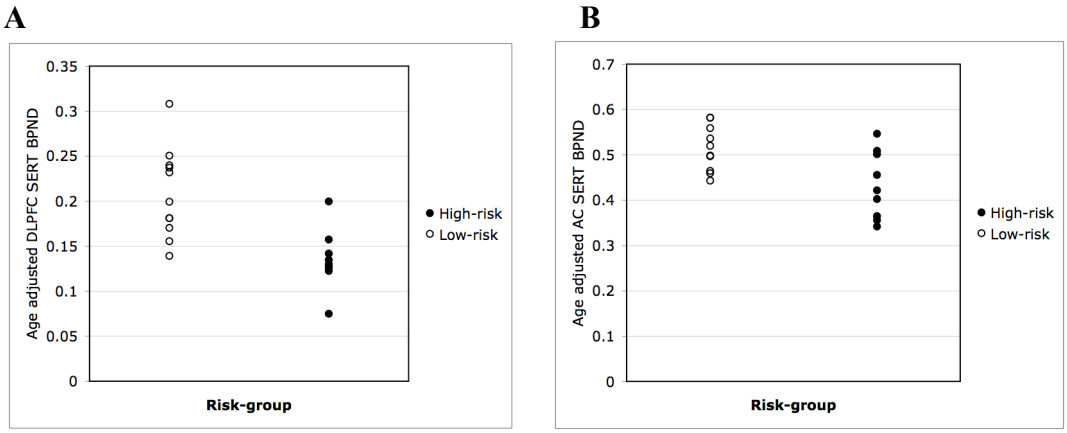


Study 3: High familial risk for mood disorder is associated with low dorsolateral prefrontal cortex serotonin transporter binding

In this study we found that healthy twins at high familial risk for mood disorders had a 35% reduction in SERT binding in dorsolateral prefrontal cortex (p=0.0018) and a 15% reduction in anterior cingulate (p=0.018), whereas, the SERT binding in subcortical regions was not significantly different. In the study 9 high-risk and 11 low-risk twins were compared. The reduction estimates correspond to the SERT binding values expected of a 30 year old.

Our data suggest that a low SERT binding in dorsolateral prefrontal cortex and anterior cingulate represent a trait marker for mood disorders.

Figure 12. Effect of familial risk of mood disorder on age-adjusted regional SERT-binding. The slope of the line for the age predictor was used to adjust the BP_{ND} to that expected of a 30 year old. DLPFC (panel A). Anterior cingulate (panel B).



Discussion

By linking personality and familial risk factors for mood disorders and in vivo characteristics of the serotonergic neurotransmission we identified a lower serotonin transporter binding in dorsolateral prefrontal cortex and anterior cingulate. We also identified a positive correlation between frontolimbic serotonin 2A receptor binding and neuroticism. Both findings may be seen as possible vulnerability markers of mood disorders.

The interpretation of these findings are discussed in paper 1, 2, and 3 in relation to the pathophysiology of mood disorders and the reader is referred to these papers for a detailed comparison with existing literature on the subject. In the following, first, the main lines in the interpretations and discussions are summarized. Second, the combined roles of SERT and serotonin 2A receptor binding in the context of mood disorders are discussed. Third, the primary methodological considerations are outlined. Finally, the replication of an association between high cortical serotonin 2A receptor binding and high body mass index (BMI) is discussed since it was not treated in detail in paper 2.

Discussion of main findings

We demonstrated in a sample of healthy volunteers a positive correlation between neuroticism and high frontolimbic serotonin 2A receptor binding (paper 1). At a causal level, we speculated that this association could be inherited and established by serotonergic modulation in early brain development and/or is as a response to low levels of synaptic serotonin possibly associated with high neuroticism. Indeed, sustained low levels of serotonin down-regulates serotonin 2A receptor levels (Cahir et al 2007; Roth et al 1998; Stockmeier and Kellar 1986).

Rather than being compensatory to low levels of serotonin, a primary high frontolimbic serotonin 2A receptor setting might also, in itself, be adverse in the context of mood disorders; serotonin 2A receptor agonism stimulates cortisol excretion (Van de Kar et al 2001), and enhanced cortical serotonin 2A signaling is accompanied by a tendency to perceive or judge an environment as risky (Weisstaub

et al 2006). Recent in vivo imaging studies support that prefrontal serotonin 2A receptor binding correlate with depression severity (Meyer et al 2003) or subclinical symptoms in recovered, non-depressed patients (Bhagwagar et al 2006). Also, personality traits, e. g. neuroticism and its constituent trait vulnerability, shape an individuals ability to cope with stress. Thus, a strong neuroticism or vulnerability component is likely to have adverse effects in the context of stress and mood disorder since stress responsiveness play a key role in the pathophysiology (Jacobs et al 2006). Accordingly, the combination of both high frontolimbic serotonin 2A receptor binding and high neuroticism may be particularly disadvantageous in the context of stress and mood disorders. We hypothesized that the coupling of high frontolimbic serotonin 2A receptor binding and high neuroticism could be accentuated by familial risk factors of mood disorders. In further support of this hypothesis the genetic overlap between neuroticism and depression in considerable, but not total, i.e. approximately 50% (Kendler et al 2006a), and neuroticism is a robust predictor of future depression (Fanous et al 2007; Kendler et al 1993b).

Study 2, confirmed our hypothesis that familial risk of mood disorder plays a role in the positive correlation between frontolimbic serotonin 2A receptor binding and neuroticism. We found that the correlation is significantly stronger in individuals with pronounced familial risk than in individuals with low familial risk. Due to the limited sample size we could not discriminate directly between genetic and environmental influences on this interaction between familial risk and neuroticism in predicting the serotonin 2A receptor binding. However, we found some support of a considerable genetic influence since the association tended to be stronger in the monozygotic high-risk group than the dizygotic high-risk group.

Particularly, early life adverse environmental factors, such as maternal stress during pregnancy or deprivation of normal parental care during infancy, may represent potent risk factors (Heim and Nemeroff 2001; Kajantie 2006; Talge et al 2007). Also, a classical twin study from our group have shown that the serotonin 2A receptor binding levels and distribution is highly heritable and only very little influenced by effects of environmental factors after early life (Pinborg et al 2007). Hence the study by Pinborg et al supports that the effect of familial risk may come about through genetic factors and early life environment.

In individuals at high familial risk of developing mood disorder, we identified lower SERT-binding in dorsolateral prefrontal cortex (DLPFC) and, to a smaller extent, in anterior cingulate. The involvement of these regions in mood disorders are strongly supported by metabolic, structural, and functional studies (Drevets et al 1997; Fitzgerald et al 2006; Mayberg et al 1997; Neumeister et al 2004; Sheline 2003; Videbech 2000).

We pointed at 3, potentially overlapping, conditions that may characterize individuals at high familial risk and lead to low serotonin transporter binding:

1) Lower serotonin levels that facilitate SERT internalization and degradation (Ramamoorthy and Blakely 1999). It is unknown if such mechanisms operate in the brain of healthy people, in response to acute and short-term decrease in synaptic serotonin levels (Praschak-Rieder et al 2005; Talbot et al 2005). Rather, down-regulation of SERT may require sustained exposure to low levels of serotonin. We speculate that the reduced DLPFC SERT binding in high-risk subjects may represent compensatory adaptations to inherited dysfunctions in serotonin homeostasis. Only when such compensatory mechanisms break down, depression occurs. Indeed, recent [^{11}C]DASB-PET studies have suggested that SERT binding in several cortical regions is elevated during depressive episodes in mood disorders (Cannon et al 2006), and that the severity of depressive symptoms correlate positively with SERT-binding (Meyer et al 2004), see table 2, p 21.

2) Reduced number of serotonergic projections to DLPFC and anterior cingulate. In support of this explanation, reductions of serotonergic axons (Austin et al 2002) and neuronal density (Rajkowska et al 1999) have been observed in DLPFC in postmortem studies of depression. Neuronal loss may also be related to loss of SERT during early brain development.

3) Decreased SERT expression determined by genetic and/or early environmental factors. In support of an impact of low SERT expression during early brain development, SERT knock-out mice show depressive-like behavior and also have a reduced serotonergic cell number and firing rate in the dorsal raphe nuclei (Lira et al 2003). Further, the low expressing ”s” variant of the SERT promoter gene is a risk factor for depression in combination with environmental stress (Caspi et al 2003;

Kendler et al 2005). This SERT-promoter-gene associated vulnerability possibly comes about through modulation of early brain development as supported by mice studies (Ansorge et al 2004; Gaspar et al 2003), yet it may also affect protein levels in the adult brain (Praschak-Rieder et al 2007), although this is disputable (Shioe et al 2003).

Thus, the reduction in DLPFC and anterior cingulate SERT binding may represent a trait marker for mood disorder that could either be established during early brain development, through a reduced number of serotonergic projections, and/or compensatory adaptations to low synaptic serotonin.

Combined roles of SERT and serotonin 2A receptor alterations in the context of mood disorders

It is presently unknown if the identified effects of familial risk, with respect to both the positive correlation between serotonin 2A receptor binding and neuroticism and the low DLPFC SERT binding, are driven by a common factor, e. g. a developmental effect established through genes or early environment. Future combined PET-measurements of the presynaptic SERT and postsynaptic serotonin 2A receptor binding within the same subjects are required and the possible additive roles in the relation to neuroticism or risk for developing mood disorder should be explored. In line with the discussion above, it is possible that a primary disturbance in developmental serotonergic modulation would influence DLPFC SERT or frontolimbic 2A directly or would cause a dysfunctional serotonin homeostasis. A lower frontal and/or limbic synaptic serotonin could contribute to both the up-regulation of serotonin 2A receptors associated with high neuroticism and the down-regulation of SERT density. Accordingly, SERT and serotonin 2A receptor adaptations are then compensatory. Unfortunately, it is not possible to probe this assumption experimentally, since synaptic levels of serotonin cannot yet be measured in vivo.

On the other hand, there may be adverse effects associated with high frontolimbic serotonin 2A receptor availability in combination with high neuroticism, and possibly low SERT availability, which could be preset genetically or via early environmental factors (Pinborg et al 2007). However, again, this remains speculative.

Alternatively, SERT could be viewed as a marker of serotonergic neurons. As such, the ratio between serotonin 2A receptor binding and SERT binding would be a measure of relative up or down-regulation of serotonin 2A receptor levels as normalized to "number of serotonergic projections". Within the frontal and anterior cingulate regions, determining such a pre and postsynaptic receptor balance might be feasible, whereas, in subcortical regions it may require extremely large sample sizes due to the noisy estimates of serotonin 2A receptor binding in low binding regions (Haugbol et al 2007). Particularly, an increased serotonin 2A/SERT ratio in frontal and cingulate regions may characterize individuals at risk for mood disorder, a hypothesis that could be evaluated in future studies.

Methodological considerations

Our results should be interpreted in the context of five potentially significant methodological considerations.

First, the pooling of frontal and limbic regions in study 1 and 2 may be problematic. Serotonin 2A receptor binding can be measured with high reproducibility in cortical regions, but less in subcortical regions, e.g. the hippocampus (Haugbol et al 2007). Hippocampus was included in the frontolimbic region due to its key role in the pathophysiology of mood disorders (Fujita et al 2000; Sheline 2003), however, the inclusion may have added noise to the dataset. Nevertheless, in a large sample of 83 subjects, such as study 1, the statistical power is sufficient (Haugbol et al 2007). Another concern when including hippocampus relate to the pathophysiology, that is, changes in serotonin 2A receptor binding associated with depression may be in opposite directions in hippocampus as compared to frontal cortex; Mintun et al (2004) reports an isolated decreased serotonin 2A receptor binding in hippocampus in patients with major depression off medication for at least 4-6 weeks. Also, several postmortem reports support a decrease in hippocampal serotonin 2A receptor binding in depression (Stockmeier 2003). Finally, animal studies support a differential regulation of serotonin 2A receptor levels induced by chronic stress, with up-regulation in frontal cortex and down-regulation in the hippocampus, only in rats prone to develop learned helplessness – a behavioral model of vulnerability to depression (Dwivedi et al 2005). However, some of these findings may be due to brain volume loss known to be a vulnerability factor in mood disorders

(Gilbertson et al 2002) rather than specific loss of serotonin 2A receptors. In study 1, in hippocampus, we could not demonstrate a negative correlation between frontolimbic 2A and neuroticism. On the contrary, the positive association was borderline significant as seen in post hoc analyses (p=0.073). Accordingly, we may have found a more significant association between serotonin 2A receptor binding and neuroticism had we excluded the hippocampus from our hypothesis. Based on the existing literature it is hard to exclude a potential contribution from hippocampal serotonin 2A receptor binding in the risk of developing mood disorders.

Second the potential relation between neuroticism and vulnerability score and physiological stress in response to emotional stress could not be directly evaluated in study 1 or 2. Unfortunately, data on e.g. cortisol waking response were not available. Therefore, the link between stress and neuroticism or vulnerability score remains speculative.

Third, with regard to the register-based studies (2 and 3), the high-risk group consisted of subjects with variable risk-load and, particularly in study 2, several aspects tended to reduce the risk. In study 2, the average age of diagnosis of the co-twin was 32.2 ± 8.8 years, with 3 affected co-twins being older than 40 years (43, 47, 54). Later age of onset would be anticipated to be associated with lower risk. Also the at-risk twins varied considerably with respect to number of years passed from age of diagnosis of the co-twins mood disorder to inclusion. The mean symptom free interval was 7.0±7.6 years. In 3 subjects more than 10 years had passed from the age of onset of the co-twin (10, 20, and 34 years). These subjects did, despite their genetic make-up, for a long time not develop mood disorders and hence may be protected. Finally, subjects volunteering for an extensive investigation program, including a PET-study, would tend to be more robust in general. Therefore, it is possible that an even more prominent association between frontolimbic serotonin 2A receptor binding and neuroticism could have been found in a high-risk group more selected towards high risk. In the next study, 3, we specifically aimed at securing a homogenous and substantial risk-load in the high-risk group. Therefore, the following inclusion criteria were applied: 1) at onset of mood disorder, the co-twin was less than 35 yrs, and 2) the number of years passed from age of onset of the co-twin to inclusion of the unaffected twin should be less than 5 years.

Fourth, we pooled individuals predisposed to mood disorder both through uni- and bipolar disorder which may tend to add to the heterogeneity of the high-risk group. In both study 1 and 2, the fraction of predisposition through bipolar co-twins was considerable (study 2: 19%, study 3: 33%). It is possible that unipolar and bipolar disorder constitute a continuous spectrum, rather than being categorically distinct (Akiskal and Benazzi 2006). More importantly, keeping our main goal in mind, there is no difference in the frequency of unipolar depression in relatives of uni- and bipolars, leaving the risk unaltered in the healthy high-risk twin group (Kutcher and Marton 1991). In addition, the register based approach identified individuals who had received a uni- or bipolar diagnosis at their first admission. Therefore, later conversion from uni- to bipolar disorder was not taken into account. Conversion can be expected in about 10-15% of the patients initially diagnosed as unipolar (Akiskal et al 1995). Accordingly, in any case, separation of uni- and bipolar risk-groups would be inappropriate. Importantly, the validity of the diagnoses at latest admission in the Danish Psychiatric Central Register is high; Kessing et al. (1998) showed that 95% of the diagnoses of affective disorder was confirmed by follow-up. Also, the correlation between age at first episode and age at first admission was high (r=0.92).

Fifth, we studied twins and, thus, the outcome may not be representative of singletons. However, several studies support that mood disorders in twins are typical to that of the general population; both monozygotic and dizygotic twins resemble the background population in their risk for mood disorders (Kendler et al 1996; Klaning et al 2004), and in their level and variability of self-reported psychiatric symptoms (Kendler et al 1995). It should be emphasized, that the studies 2 and 3 were not designed as traditional twin studies, to answer questions about the relative influence of genetic versus early environmental factors. Rather, our aim was to identify healthy subjects at high or low risk for mood disorder, and the twins (even the dizygotics) served that purpose better than other relatives to patients with mood disorders. Yet, it is not clear whether these results can be extrapolated to other ethnic or geographical populations.

Body mass index (BMI) and elevated frontolimbic serotonin 2A receptor binding

In study 2, BMI was positively correlated with frontolimbic serotonin 2A receptor binding within a normal range of BMI. This is an independent replication of an earlier finding in a sample of 49 subjects (Adams et al 2004). This coupling might reflect that serotonergic neurotransmission is involved in appetite regulation, eating disorders (Kaye et al 2005), and obesity. In particular, functional polymorphisms of the serotonin 2A receptor promoter gene has been associated with obesity (Rosmond et al 2002). Animal studies show that serotonin depletion is associated with overeating and weight gain in (Saller and Stricker 1976; Waldbillig et al 1981) and decreased synaptic serotonin may up-regulate serotonin 2A receptors. Further, common genetic factors could predispose to both obesity and major depression. Indeed, obesity is a risk factor for later development of depression in women (Anderson et al 2007), modest associations between neuroticism and BMI within the normal range has been reported in women (Faith et al 2001), and obesity and depression share pathophysiological features, e. g. in terms of dysregulation of the HPA-axis resulting in cortisol hypersecretion (Bornstein et al 2006). Also, both conditions respond to SSRI treatment. We were, however, not able to identify any significant interactions between BMI and familial risk of mood disorder ($p=0.91$), or BMI and neuroticism ($p=0.41$) in a model adjusting for age, neuroticism, and the neuroticism-risk interaction, degrees of freedom: 31 (data not shown).

Since we quantified the specific serotonin 2A receptor binding in steady-state relative to the concentration of parent compound in plasma it is not likely that metabolic factors would have biased the association between serotonin 2A receptor binding and BMI.

Conclusions

Based on in vivo PET-measurements in humans, this work identifies a characteristic pattern in the serotonergic neurotransmission that may serve as a neurobiological link between personality and familial risk factors for mood disorder and the actual development of the disorder.

For the first time, a lower SERT binding in the dorsolateral prefrontal cortex and anterior cingulate is linked to familial risk, and a higher frontolimbic serotonin 2A receptor binding is linked to the personality risk factor neuroticism. Neuroticism may simply reflect genetic risk factors of developing mood disorder, and as such index the genetic risk. But in addition, it may in itself mediate adverse effects by enhancing the impact of environmental risk factors, e.g. potentially stressful experiences in a person's life. Indeed, we found support for an interaction between neuroticism and familial risk in the association with frontolimbic serotonin 2A receptor binding.

The identified serotonin 2A receptor and SERT trait pattern of risk for mood disorders may be established either during early brain development, through a reduced number of serotonergic projections, and/or compensatory adaptations to low synaptic serotonin.

Taken together, these findings demonstrate that alterations in serotonergic neurotransmission are present in mentally healthy, asymptomatic individuals prone to develop mood disorder and, thus, may support our understanding of how familial risk predispose to mood disorders. However, only longitudinal follow-up studies can clarify whether these identified serotonergic trait characteristics actually translate into the clinical expression of mood disorder. Such studies might also uncover additional relevant interactions between risk factors for mood disorder and help elucidating why some individuals at high risk develop mood disorders and others do not.

By understanding better the pathophysiology of mood disorders, these findings may contribute to develop better treatment, earlier intervention, and maybe even preventive strategies for persons at high risk for developing mood disorders.

Research perspectives

Longitudinal, prospective studies in high-risk cohorts with clinical follow-up are warranted to characterize those individuals that develop mood disorders later on. Such studies should aim at the following:

1. Examine if high frontolimbic serotonin 2A receptor binding in combination with high neuroticism score predicts which at-risk individuals develop a mood disorder.
2. Examine if a premorbid low DLPFC and anterior cingulate binding is predictive of individuals that later present with a mood disorder.
3. Elucidate whether a lower DLPFC SERT binding, in individuals at high-risk of developing mood disorders, represents a compensatory state, by scheduling repeated scanning, before treatment is initiated, of those high-risk individuals (and a control group) that develop mood disorder.
4. Enable discrimination between genetic and environmental factors by including a large cohort of high-risk twins, and ideally include both twin pairs.
5. Allow continued focus on the complex mechanisms likely to interact in the translation of familial risk to the clinical expression of mood disorders by ensuring data collection on personality, state measures on mood, and cortisol secretion e.g. wakening response, measures of perceived stress, influences of gender or endogenous sex hormones, biomarkers of neurodegeneration or neuroprotection, e. g. BDNF, and genes of relevance to mood disorders, neurodegeneration or neuroprotection.
6. Examine “protective” or “opportunity” genes that are most likely to be in play in the context of mood disorders in parallel with “vulnerability” genes, presumably, penetrating through interactions with beneficial environmental factors. Such beneficial gene by environment effects in the context of mood disorders might be studied in the longitudinal set-up by focusing on those twins or siblings who despite their risk have not developed depression in e.g. 10 years. Such studies would directly shed light on potential preventive strategies.

As an alternative strategy to twin cohorts, it may be beneficial to select a high-risk group based on family history of early onset, recurrent depression in two or more generations (Weissman et al 2005). Denmark, with its 5 million inhabitants, is hardly ideal for large scale longitudinal twin studies. With long follow-up periods high prevalence rates of mood disorders can be expected in relatives of both unipolar and bipolar patients. With 20 years follow-up up to 80% of the offspring of depressed patients may develop a mood disorder (Weissman et al 2006). With 5 years of follow-up of offspring of bipolar parents up to 40 % may develop a mood disorder (Hillegers et al 2005).

Combined PET-studies on pre and postsynaptic serotonin receptor binding in the same subjects will be needed to study the associations between SERT and postsynaptic serotonin receptor binding in vivo and possible additive roles in the relation to neuroticism and/or risk of developing depression. Postsynaptic receptors of interest in this regard would be the serotonin 2A receptor and the serotonin 1A receptor.

Methodological developments that would have major impact on the possibility to study the in vivo dynamics of serotonergic neurotransmission and the relation to psychiatric disorders or neurodegenerative processes in humans would be:

1. Implementation of high resolution PET, allowing valid evaluation of smaller brain regions such as hypothalamus, amygdala, and hippocampus.
2. Development of a PET/MR scanner.
3. New tracers:
    - a tracer suitable for SERT imaging in all cortical regions.
    - a tracer suitable for measurements of serotonin 2A receptor binding in subcortical regions.
    - a tracer sensitive of endogenous serotonin release.
    - tracers suitable for new targets, e.g. the serotonin 4 receptor

4. Alternative methods for evaluation of endogenous levels of serotonin in vivo in humans, ideally non-invasive.

References

Adams KH, Pinborg LH, Svarer C, et al (2004): A database of [(18)F]-altanserin binding to 5-HT(2A) receptors in normal volunteers: normative data and relationship to physiological and demographic variables. *Neuroimage* 21:1105-13.

Akiskal HS, Benazzi F (2006): The DSM-IV and ICD-10 categories of recurrent [major] depressive and bipolar II disorders: evidence that they lie on a dimensional spectrum. *J Affect Disord* 92:45-54.

Akiskal HS, Maser JD, Zeller PJ, et al (1995): Switching from 'unipolar' to bipolar II. An 11-year prospective study of clinical and temperamental predictors in 559 patients. *Arch Gen Psychiatry* 52:114-23.

Anand A, Li Y, Wang Y, et al (2005): Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. *Biol Psychiatry* 57:1079-88.

Anderson SE, Cohen P, Naumova EN, Jacques PF, Must A (2007): Adolescent obesity and risk for subsequent major depressive disorder and anxiety disorder: prospective evidence. *Psychosom Med* 69:740-7.

Ansorge MS, Zhou M, Lira A, Hen R, Gingrich JA (2004): Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science* 306:879-81.

Arango V, Underwood MD, Boldrini M, et al (2001): Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology* 25:892-903.

Arango V, Underwood MD, Mann JJ (1997): Postmortem findings in suicide victims. Implications for in vivo imaging studies. *Ann N Y Acad Sci* 836:269-87.

Attar-Levy D, Martinot JL, Blin J, et al (1999): The cortical serotonin2 receptors studied with positron-emission tomography and [18F]-setoperone during depressive illness and antidepressant treatment with clomipramine. *Biol Psychiatry* 45:180-6.

Austin MC, Whitehead RE, Edgar CL, Janosky JE, Lewis DA (2002): Localized decrease in serotonin transporter-immunoreactive axons in the prefrontal cortex of depressed subjects committing suicide. *Neuroscience* 114:807-15.

Barnes NM, Sharp T (1999): A review of central 5-HT receptors and their function. *Neuropharmacology* 38:1083-152.

Barr CS, Newman TK, Schwandt M, et al (2004): Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. *Proc Natl Acad Sci U S A* 101:12358-63.

Benmansour S, Owens WA, Cecchi M, Morilak DA, Frazer A (2002): Serotonin clearance in vivo is altered to a greater extent by antidepressant-induced downregulation of the serotonin transporter than by acute blockade of this transporter. *J Neurosci* 22:6766-72.

Bhagwagar Z, Hafizi S, Cowen PJ (2003): Increase in concentration of waking salivary cortisol in recovered patients with depression. *Am J Psychiatry* 160:1890-1.

Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P (2006): Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL 100,907. *Am J Psychiatry* 163:1580-7.

Bhagwagar Z, Whale R, Cowen PJ (2002): State and trait abnormalities in serotonin function in major depression. *Br J Psychiatry* 180:24-8.

Biver F, Wikler D, Lotstra F, Damhaut P, Goldman S, Mendlewicz J (1997): Serotonin 5-HT2 receptor imaging in major depression: focal changes in orbito-insular cortex. *Br J Psychiatry* 171:444-8.

Blakely RD, De Felice LJ, Hartzell HC (1994): Molecular physiology of norepinephrine and serotonin transporters. *J Exp Biol* 196:263-81.

Blier P, de Montigny C, Chaput Y (1990): A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. *J Clin Psychiatry* 51 Suppl:14-20; discussion 21.

Bornstein SR, Schuppenies A, Wong ML, Licinio J (2006): Approaching the shared biology of obesity and depression: the stress axis as the locus of gene-environment interactions. *Mol Psychiatry* 11:892-902.

Brummett BH, Boyle SH, Siegler IC, et al (2007): Effects of Environmental Stress and Gender on Associations among Symptoms of Depression and the Serotonin Transporter Gene Linked Polymorphic Region (5-HTTLPR). *Behav Genet.*

Caetano SC, Kaur S, Brambilla P, et al (2006): Smaller cingulate volumes in unipolar depressed patients. *Biol Psychiatry* 59:702-6.

Cahir M, Ardis T, Reynolds GP, Cooper SJ (2007): Acute and chronic tryptophan depletion differentially regulate central 5-HT1A and 5-HT 2A receptor binding in the rat. *Psychopharmacology (Berl)* 190:497-506.

Cannon DM, Ichise M, Fromm SJ, et al (2006): Serotonin transporter binding in bipolar disorder assessed using [11C]DASB and positron emission tomography. *Biol Psychiatry* 60:207-17.

Cannon DM, Ichise M, Rollis D, et al (2007): Elevated Serotonin Transporter Binding in Major Depressive Disorder Assessed Using Positron Emission Tomography and [(11)C]DASB; Comparison with Bipolar Disorder. *Biol Psychiatry*.

Caspi A, Roberts BW, Shiner RL (2005): Personality development: stability and change. *Annu Rev Psychol* 56:453-84.

Caspi A, Sugden K, Moffitt TE, et al (2003): Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 301:386-9.

Celada P, Puig M, Amargos-Bosch M, Adell A, Artigas F (2004): The therapeutic role of 5-HT1A and 5-HT2A receptors in depression. *J Psychiatry Neurosci* 29:252-65.

Cortes R, Soriano E, Pazos A, Probst A, Palacios JM (1988): Autoradiography of antidepressant binding sites in the human brain: localization using [3H]imipramine and [3H]paroxetine. *Neuroscience* 27:473-96.

Costa PT, McCrae RR (1992): *Professional manual for Revised NEO Personality Inventory*: Odessa FL: Psychological Assessment Resources.

D'Haenen H, Bossuyt A, Mertens J, Bossuyt-Piron C, Gijsemans M, Kaufman L (1992): SPECT imaging of serotonin2 receptors in depression. *Psychiatry Res* 45:227-37.

Dahlstrom M, Ahonen A, Ebeling H, Torniainen P, Heikkila J, Moilanen I (2000): Elevated hypothalamic/midbrain serotonin (monoamine) transporter availability in depressive drug-naive children and adolescents. *Mol Psychiatry* 5:514-22.

Drevets WC (2000): Neuroimaging studies of mood disorders. *Biol Psychiatry* 48:813-29.

Drevets WC, Price JL, Simpson JR, Jr., et al (1997): Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 386:824-7.

Drevets WC, Thase ME, Moses-Kolko EL, et al (2007): Serotonin-1A receptor imaging in recurrent depression: replication and literature review. *Nucl Med Biol* 34:865-77.

Dwivedi Y, Mondal AC, Payappagoudar GV, Rizavi HS (2005): Differential regulation of serotonin (5HT)2A receptor mRNA and protein levels after single and repeated stress in rat brain: role in learned helplessness behavior. *Neuropharmacology* 48:204-14.

Eysenck H, Eysenck S (1991): *Manual of the Eysenck Personality Scales (EPS Adult)*. London, UK: Hodder & Stoughton.

Faith MS, Flint J, Fairburn CG, Goodwin GM, Allison DB (2001): Gender differences in the relationship between personality dimensions and relative body weight. *Obes Res* 9:647-50.

Fanous A, Gardner CO, Prescott CA, Cancro R, Kendler KS (2002): Neuroticism, major depression and gender: a population-based twin study. *Psychol Med* 32:719-28.

Fanous AH, Neale MC, Aggen SH, Kendler KS (2007): A longitudinal study of personality and major depression in a population-based sample of male twins. *Psychol Med* 37:1163-72.

Fitzgerald PB, Oxley TJ, Laird AR, Kulkarni J, Egan GF, Daskalakis ZJ (2006): An analysis of functional neuroimaging studies of dorsolateral prefrontal cortical activity in depression. *Psychiatry Res* 148:33-45.

Frokjaer V, Pinborg L, Madsen J, et al (2008): Evaluation of the serotonin transporter ligand [^{123}I]ADAM for SPECT studies in humans. *J Nucl Med*. In press.

Fujita M, Charney DS, Innis RB (2000): Imaging serotonergic neurotransmission in depression: hippocampal pathophysiology may mirror global brain alterations. *Biol Psychiatry* 48:801-12.

Gaspar P, Cases O, Maroteaux L (2003): The developmental role of serotonin: news from mouse molecular genetics. *Nat Rev Neurosci* 4:1002-12.

Gilbertson MW, Shenton ME, Ciszewski A, et al (2002): Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat Neurosci* 5:1242-7.

Gray JA, Roth BL (2001): Paradoxical trafficking and regulation of 5-HT(2A) receptors by agonists and antagonists. *Brain Res Bull* 56:441-51.

Harvald B, Hauge G, Kyvik KO, Christensen K, Skytthe A, Holm NV (2004): The danish twin registry: past and present. *Twin Res* 7:318-35.

Hasin DS, Goodwin RD, Stinson FS, Grant BF (2005): Epidemiology of major depressive disorder: results from the National Epidemiologic Survey on Alcoholism and Related Conditions. *Arch Gen Psychiatry* 62:1097-106.

Haugbol S, Pinborg LH, Arfan HM, et al (2007): Reproducibility of 5-HT(2A) receptor measurements and sample size estimations with [(18)F]altanserin PET using a bolus/infusion approach. *Eur J Nucl Med Mol Imaging* 34:910-915.

Heim C, Nemeroff CB (2001): The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* 49:1023-39.

Hillegers MH, Reichart CG, Wals M, Verhulst FC, Ormel J, Nolen WA (2005): Five-year prospective outcome of psychopathology in the adolescent offspring of bipolar parents. *Bipolar Disord* 7:344-50.

Holmans P, Weissman MM, Zubenko GS, et al (2007): Genetics of recurrent early-onset major depression (GenRED): final genome scan report. *Am J Psychiatry* 164:248-58.

Huang Y, Hwang DR, Narendran R, et al (2002): Comparative evaluation in nonhuman primates of five PET radiotracers for imaging the serotonin transporters: [11C]McN 5652, [11C]ADAM, [11C]DASB, [11C]DAPA, and [11C]AFM. *J Cereb Blood Flow Metab* 22:1377-98.

Ichimiya T, Suhara T, Sudo Y, et al (2002): Serotonin transporter binding in patients with mood disorders: a PET study with [11C](+)McN5652. *Biol Psychiatry* 51:715-22.

Ichise M, Liow JS, Lu JQ, et al (2003): Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J Cereb Blood Flow Metab* 23:1096-112.

Jacobs N, Kenis G, Peeters F, Derom C, Vlietinck R, van Os J (2006): Stress-related negative affectivity and genetically altered serotonin transporter function: evidence of synergism in shaping risk of depression. *Arch Gen Psychiatry* 63:989-96.

Jang KL, Livesley WJ, Vernon PA (1996): Heritability of the big five personality dimensions and their facets: a twin study. *J Pers* 64:577-91.

Jokela M, Keltikangas-Jarvinen L, Kivimaki M, et al (2007): Serotonin receptor 2A gene and the influence of childhood maternal nurturance on adulthood depressive symptoms. *Arch Gen Psychiatry* 64:356-60.

Jonsson EG, Nothen MM, Gustavsson JP, et al (2001): No association between serotonin 2A receptor gene variants and personality traits. *Psychiatr Genet* 11:11-7.

Kajantie E (2006): Fetal origins of stress-related adult disease. *Ann N Y Acad Sci* 1083:11-27.

Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM (2002): Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res* 109:143-8.

Kaye WH, Frank GK, Bailer UF, et al (2005): Serotonin alterations in anorexia and bulimia nervosa: new insights from imaging studies. *Physiol Behav* 85:73-81.

Kendler KS, Gardner CO, Prescott CA (1999): Clinical characteristics of major depression that predict risk of depression in relatives. *Arch Gen Psychiatry* 56:322-7.

Kendler KS, Gatz M, Gardner CO, Pedersen NL (2006a): Personality and major depression: a Swedish longitudinal, population-based twin study. *Arch Gen Psychiatry* 63:1113-20.

Kendler KS, Gatz M, Gardner CO, Pedersen NL (2006b): A Swedish national twin study of lifetime major depression. *Am J Psychiatry* 163:109-14.

Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B (2005): The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch Gen Psychiatry* 62:529-35.

Kendler KS, Martin NG, Heath AC, Eaves LJ (1995): Self-report psychiatric symptoms in twins and their nontwin relatives: are twins different? *Am J Med Genet* 60:588-91.

Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ (1993a): The lifetime history of major depression in women. Reliability of diagnosis and heritability. *Arch Gen Psychiatry* 50:863-70.

Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ (1993b): A longitudinal twin study of personality and major depression in women. *Arch Gen Psychiatry* 50:853-62.

Kendler KS, Pedersen NL, Farahmand BY, Persson PG (1996): The treated incidence of psychotic and affective illness in twins compared with population expectation: a study in the Swedish Twin and Psychiatric Registries. *Psychol Med* 26:1135-44.

Kessing LV (1998): Validity of diagnoses and other clinical register data in patients with affective disorder. *Eur Psychiatry* 13:392-8.

Kim JS, Ichise M, Sangare J, Innis RB (2006): PET imaging of serotonin transporters with [11C]DASB: test-retest reproducibility using a multilinear reference tissue parametric imaging method. *J Nucl Med* 47:208-14.

Kish SJ, Furukawa Y, Chang LJ, et al (2005): Regional distribution of serotonin transporter protein in postmortem human brain: is the cerebellum a SERT-free brain region? *Nucl Med Biol* 32:123-8.

Klaassen T, Riedel WJ, van Someren A, Deutz NE, Honig A, van Praag HM (1999): Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. *Biol Psychiatry* 46:489-97.

Klaning U, Laursen TM, Licht RW, Kyvik KO, Skytthe A, Mortensen PB (2004): Is the risk of bipolar disorder in twins equal to the risk in singletons? A nationwide register-based study. *J Affect Disord* 81:141-5.

Klimek V, Roberson G, Stockmeier CA, Ordway GA (2003): Serotonin transporter and MAO-B levels in monoamine nuclei of the human brainstem are normal in major depression. *J Psychiatr Res* 37:387-97.

Kristiansen H, Elfving B, Plenge P, Pinborg LH, Gillings N, Knudsen GM (2005): Binding characteristics of the 5-HT2A receptor antagonists altanserin and MDL 100907. *Synapse* 58:249-57.

Kusumi I, Suzuki K, Sasaki Y, Kameda K, Sasaki T, Koyama T (2002): Serotonin 5-HT(2A) receptor gene polymorphism, 5-HT(2A) receptor function and personality traits in healthy subjects: a negative study. *J Affect Disord* 68:235-41.

Kutcher S, Marton P (1991): Affective disorders in first-degree relatives of adolescent onset bipolars, unipolars, and normal controls. *J Am Acad Child Adolesc Psychiatry* 30:75-8.

Kyvik KO, Christensen K, Skytthe A, Harvald B, Holm NV (1996): The Danish Twin Register. *Dan Med Bull* 43:467-70.

Lammertsma AA, Hume SP (1996): Simplified reference tissue model for PET receptor studies. *Neuroimage* 4:153-8.

Lang UE, Hellweg R, Gallinat J (2004): BDNF serum concentrations in healthy volunteers are associated with depression-related personality traits. *Neuropsychopharmacology* 29:795-8.

Levinson DF (2006): The genetics of depression: a review. *Biol Psychiatry* 60:84-92.

Lira A, Zhou M, Castanon N, et al (2003): Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. *Biol Psychiatry* 54:960-71.

Lucas G, Rymar VV, Du J, et al (2007): Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* 55:712-25.

Lyons MJ, Eisen SA, Goldberg J, et al (1998): A registry-based twin study of depression in men. *Arch Gen Psychiatry* 55:468-72.

Malison RT, Price LH, Berman R, et al (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* 44:1090-8.

Mann JJ, Huang YY, Underwood MD, et al (2000): A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. *Arch Gen Psychiatry* 57:729-38.

Mannie ZN, Harmer CJ, Cowen PJ (2007): Increased waking salivary cortisol levels in young people at familial risk of depression. *Am J Psychiatry* 164:617-21.

Mayberg HS, Brannan SK, Mahurin RK, et al (1997): Cingulate function in depression: a potential predictor of treatment response. *Neuroreport* 8:1057-61.

McGuffin P, Katz R, Rutherford J (1991): Nature, nurture and depression: a twin study. *Psychol Med* 21:329-35.

McMahon FJ, Buervenich S, Charney D, et al (2006): Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *Am J Hum Genet* 78:804-14.

Meltzer CC, Price JC, Mathis CA, et al (1999): PET imaging of serotonin type 2A receptors in late-life neuropsychiatric disorders. *Am J Psychiatry* 156:1871-8.

Messa C, Colombo C, Moresco RM, et al (2003): 5-HT(2A) receptor binding is reduced in drug-naive and unchanged in SSRI-responder depressed patients compared to healthy controls: a PET study. *Psychopharmacology (Berl)* 167:72-8.

Meyer JH (2007): Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. *J Psychiatry Neurosci* 32:86-102.

Meyer JH, Houle S, Sagrati S, et al (2004): Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry* 61:1271-9.

Meyer JH, Kapur S, Houle S, et al (1999): Prefrontal cortex 5-HT2 receptors in depression: an [18F]setoperone PET imaging study. *Am J Psychiatry* 156:1029-34.

Meyer JH, McMain S, Kennedy SH, et al (2003): Dysfunctional attitudes and 5-HT2 receptors during depression and self-harm. *Am J Psychiatry* 160:90-9.

Meyer JH, Wilson AA, Ginovart N, et al (2001): Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [(11)C]DASB PET imaging study. *Am J Psychiatry* 158:1843-9.

Mintun MA, Sheline YI, Moerlein SM, Vlassenko AG, Huang Y, Snyder AZ (2004): Decreased hippocampal 5-HT2A receptor binding in major depressive disorder: in vivo measurement with [18F]altanserin positron emission tomography. *Biol Psychiatry* 55:217-24.

Modell S, Lauer CJ, Schreiber W, Huber J, Krieg JC, Holsboer F (1998): Hormonal response pattern in the combined DEX-CRH test is stable over time in subjects at high familial risk for affective disorders. *Neuropsychopharmacology* 18:253-62.

Munk-Jorgensen P, Mortensen PB (1997): The Danish Psychiatric Central Register. *Dan Med Bull* 44:82-4.

Murray CJ, Lopez AD (1996): Evidence-based health policy--lessons from the Global Burden of Disease Study. *Science* 274:740-3.

Neumeister A, Nugent AC, Waldeck T, et al (2004): Neural and behavioral responses to tryptophan depletion in unmedicated patients with remitted major depressive disorder and controls. *Arch Gen Psychiatry* 61:765-73.

Oquendo MA, Hastings RS, Huang YY, et al (2007): Brain serotonin transporter binding in depressed patients with bipolar disorder using positron emission tomography. *Arch Gen Psychiatry* 64:201-8.

Oswald P, Souery D, Massat I, et al (2003): Lack of association between the 5HT2A receptor polymorphism (T102C) and unipolar affective disorder in a multicentric European study. *Eur Neuropsychopharmacol* 13:365-8.

Parsey RV, Hastings RS, Oquendo MA, et al (2006a): Lower serotonin transporter binding potential in the human brain during major depressive episodes. *Am J Psychiatry* 163:52-8.

Parsey RV, Oquendo MA, Ogden RT, et al (2006b): Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. *Biol Psychiatry* 59:106-13.

Pazos A, Probst A, Palacios JM (1987): Serotonin receptors in the human brain--IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience* 21:123-39.

Pinborg L, Arfan H, Haugbol S, et al (2007): The density of 5-HT_{2A} receptors in the human brain is strongly genetically determined. *Neuroimage* doi:10.1016/j.neuroimage.2007.09.019.

Pinborg LH, Adams KH, Svarer C, et al (2003): Quantification of 5-HT2A receptors in the human brain using [18F]altanserin-PET and the bolus/infusion approach. *J Cereb Blood Flow Metab* 23:985-96.

Pini S, de Queiroz V, Pagnin D, et al (2005): Prevalence and burden of bipolar disorders in European countries. *Eur Neuropsychopharmacol* 15:425-34.

Pirker W, Asenbaum S, Hauk M, et al (2000): Imaging serotonin and dopamine transporters with 123I-beta-CIT SPECT: binding kinetics and effects of normal aging. *J Nucl Med* 41:36-44.

Portella MJ, Harmer CJ, Flint J, Cowen P, Goodwin GM (2005): Enhanced early morning salivary cortisol in neuroticism. *Am J Psychiatry* 162:807-9.

Praschak-Rieder N, Kennedy J, Wilson AA, et al (2007): Novel 5-HTTLPR Allele Associates with Higher Serotonin Transporter Binding in Putamen: A [(11)C] DASB Positron Emission Tomography Study. *Biol Psychiatry*.

Praschak-Rieder N, Wilson AA, Hussey D, et al (2005): Effects of tryptophan depletion on the serotonin transporter in healthy humans. *Biol Psychiatry* 58:825-30.

Price JC, Lopresti BJ, Meltzer CC, et al (2001): Analyses of [(18)F]altanserin bolus injection PET data. II: consideration of radiolabeled metabolites in humans. *Synapse* 41:11-21.

Quintin P, Benkelfat C, Launay JM, et al (2001): Clinical and neurochemical effect of acute tryptophan depletion in unaffected relatives of patients with bipolar affective disorder. *Biol Psychiatry* 50:184-90.

Rajkowska G, Halaris A, Selemon LD (2001): Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biol Psychiatry* 49:741-52.

Rajkowska G, Miguel-Hidalgo JJ, Wei J, et al (1999): Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry* 45:1085-98.

Ramamoorthy S, Blakely RD (1999): Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. *Science* 285:763-6.

Reimold M, Smolka MN, Schumann G, et al (2007): Midbrain serotonin transporter binding potential measured with [11C]DASB is affected by serotonin transporter genotype. *J Neural Transm* 114:635-9.

Rios M, Lambe EK, Liu R, et al (2006): Severe deficits in 5-HT2A -mediated neurotransmission in BDNF conditional mutant mice. *J Neurobiol* 66:408-20.

Rosmond R, Bouchard C, Bjorntorp P (2002): Increased abdominal obesity in subjects with a mutation in the 5-HT(2A) receptor gene promoter. *Ann N Y Acad Sci* 967:571-5.

Roth BL, Berry SA, Kroeze WK, Willins DL, Kristiansen K (1998): Serotonin 5-HT2A receptors: molecular biology and mechanisms of regulation. *Crit Rev Neurobiol* 12:319-38.

Saller CF, Stricker EM (1976): Hyperphagia and increased growth in rats after intraventricular injection of 5,7-dihydroxytryptamine. *Science* 192:385-7.

Sheline YI (2003): Neuroimaging studies of mood disorder effects on the brain. *Biol Psychiatry* 54:338-52.

Shifman S, Bhomra A, Smiley S, et al (2007): A whole genome association study of neuroticism using DNA pooling. *Mol Psychiatry*.

Shioe K, Ichimiya T, Suhara T, et al (2003): No association between genotype of the promoter region of serotonin transporter gene and serotonin transporter binding in human brain measured by PET. *Synapse* 48:184-8.

Skovdahl-Hansen H, Mortensen, EL., Scioetz, HK. (2004): *Dokumentation for den danske udgave af NEO PI-R og NEO PI-R Kort Version.* Copenhagen, Denmark: Dansk psykologisk forlag.

Stockmeier CA (2003): Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. *J Psychiatr Res* 37:357-73.

Stockmeier CA, Kellar KJ (1986): In vivo regulation of the serotonin-2 receptor in rat brain. *Life Sci* 38:117-27.

Stockmeier CA, Shapiro LA, Dilley GE, Kolli TN, Friedman L, Rajkowska G (1998): Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. *J Neurosci* 18:7394-401.

Sullivan PF, Neale MC, Kendler KS (2000): Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry* 157:1552-62.

Svarer C, Madsen K, Hasselbalch SG, et al (2005): MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage* 24:969-79.

Tafet GE, Idoyaga-Vargas VP, Abulafia DP, et al (2001): Correlation between cortisol level and serotonin uptake in patients with chronic stress and depression. *Cogn Affect Behav Neurosci* 1:388-93.

Talbot PS, Frankle WG, Hwang DR, et al (2005): Effects of reduced endogenous 5-HT on the in vivo binding of the serotonin transporter radioligand 11C-DASB in healthy humans. *Synapse* 55:164-75.

Talge NM, Neal C, Glover V (2007): Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry* 48:245-61.

Terracciano A, Abdel-Khalek AM, Adam N, et al (2005): National character does not reflect mean personality trait levels in 49 cultures. *Science* 310:96-100.

Tochigi M, Umekage T, Kato C, et al (2005): Serotonin 2A receptor gene polymorphism and personality traits: no evidence for significant association. *Psychiatr Genet* 15:67-9.

Trajkovska V, Vinberg M, Aznar S, Knudsen GM, Kessing LV (2007): Whole blood BDNF levels in healthy twins discordant for affective disorder: Association to life events and neuroticism. *J Affect Disord*.

Van de Kar LD, Javed A, Zhang Y, Serres F, Raap DK, Gray TS (2001): 5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate hypothalamic CRF and oxytocin-expressing cells. *J Neurosci* 21:3572-9.

van Dyck CH, Malison RT, Seibyl JP, et al (2000): Age-related decline in central serotonin transporter availability with [(123)I]beta-CIT SPECT. *Neurobiol Aging* 21:497-501.

van Dyck CH, Malison RT, Staley JK, et al (2004): Central serotonin transporter availability measured with [123I]beta-CIT SPECT in relation to serotonin transporter genotype. *Am J Psychiatry* 161:525-31.

Varnas K, Halldin C, Hall H (2004): Autoradiographic distribution of serotonin transporters and receptor subtypes in human brain. *Hum Brain Mapp* 22:246-60.

Videbech P (2000): PET measurements of brain glucose metabolism and blood flow in major depressive disorder: a critical review. *Acta Psychiatr Scand* 101:11-20.

Waldbillig RJ, Bartness TJ, Stanley BG (1981): Increased food intake, body weight, and adiposity in rats after regional neurochemical depletion of serotonin. *J Comp Physiol Psychol* 95:391-405.

Weissman MM, Wickramaratne P, Nomura Y, Warner V, Pilowsky D, Verdeli H (2006): Offspring of depressed parents: 20 years later. *Am J Psychiatry* 163:1001-8.

Weissman MM, Wickramaratne P, Nomura Y, et al (2005): Families at high and low risk for depression: a 3-generation study. *Arch Gen Psychiatry* 62:29-36.

Weisstaub NV, Zhou M, Lira A, et al (2006): Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. *Science* 313:536-40.

WHO (1967): *Manual of the International Classification of Diseases, Eight Revision (ICD-8)*. Genova, Switzerland.

WHO (2005): *International Classification of Diseases, 10th Revision (ICD-10)*. Genova, Switzerland.

Willeit M, Praschak-Rieder N, Neumeister A, et al (2000): [123I]-beta-CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. *Biol Psychiatry* 47:482-9.

Willeit M, Stastny J, Pirker W, et al (2001): No evidence for in vivo regulation of midbrain serotonin transporter availability by serotonin transporter promoter gene polymorphism. *Biol Psychiatry* 50:8-12.

Yatham LN, Liddle PF, Shiah IS, et al (2000): Brain serotonin2 receptors in major depression: a positron emission tomography study. *Arch Gen Psychiatry* 57:850-8.

Zammit S, Owen MJ (2006): Stressful life events, 5-HTT genotype and risk of depression. *Br J Psychiatry* 188:199-201.

Two recent studies were added to Table 2 after the thesis was handed in January 2th 2008

Bhagwagar Z, Murthy N, Selvaraj S, *et al* (2007): 5-HTT Binding in Recovered Depressed Patients and Healthy Volunteers: A Positron Emission Tomography Study With [11C]DASB. *Am J Psychiatry* **164**:1858-65.

Reimold M, Batra A, Knobel A, *et al* (2008): Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [(11)C]DASB PET study. *Mol Psychiatry,* print copy in press, (orginally published online February 12, 2008, at www.nature.com/mp/journal/ vaop/ncurrent/abs/4002149a.html).

Appendices

1. Frontolimbic serotonin 2A receptor binding in healthy subjects is associated with personality risk factors for affective disorder.

2. Frontolimbic serotonin 2A receptor binding, neuroticism, and familial risk for mood disorder.

3. High familial risk for mood disorder is associated with low dorsolateral prefrontal cortex serotonin transporter binding.

Paper 1

Frontolimbic Serotonin 2A Receptor Binding in Healthy Subjects Is Associated with Personality Risk Factors for Affective Disorder

Vibe G. Frokjaer, Erik L. Mortensen, Finn Å. Nielsen, Steven Haugbol, Lars H. Pinborg, Karen H. Adams, Claus Svarer, Steen G. Hasselbalch, Soeren Holm, Olaf B. Paulson, and Gitte M. Knudsen

Background: Serotonergic dysfunction has been associated with affective disorders. High trait neuroticism, as measured on personality inventories, is a risk factor for major depression. In this study we investigated whether neuroticism is associated with serotonin 2A receptor binding in brain regions of relevance for affective disorders.

Methods: Eighty-three healthy volunteers completed the standardized personality questionnaire NEO-PI-R (Revised NEO Personality Inventory) and underwent [^{18}F]altanserin positron emission tomography imaging for assessment of serotonin 2A receptor binding. The correlation between the neuroticism score and frontolimbic serotonin 2A receptor binding was evaluated by multiple linear regression analysis with adjustment for age and gender.

Results: Neuroticism correlated positively with frontolimbic serotonin 2A receptor binding [$r(79)$ .24, p .028]. Post hoc analysis of the contributions from the six constituent traits of neuroticism showed that the correlation was primarily driven by two of them: vulnerability and anxiety. Indeed, vulnerability, defined as a person's difficulties in coping with stress, displayed the strongest positive correlation, which remained significant after correction for multiple comparisons (r .35, p .009).

Conclusions: In healthy subjects the personality dimension neuroticism and particularly its constituent trait, vulnerability, are positively associated with frontolimbic serotonin 2A binding. Our findings point to a neurobiological link between personality risk factors for affective disorder and the serotonergic transmitter system and identify the serotonin 2A receptor as a biomarker for vulnerability to affective disorder.

Key Words: Affective disorder, anxiety, depression, imaging, mood disorder, NEO-PI-R, neuroticism, personality, PET, serotonin, 2A, 5HT, 5HT2A

Serotonergic neurotransmission is involved in both behavioral characteristics, such as anxiety, impulsiveness, and aggression, and in neuropsychiatric disorders, including major depression, anxiety disorders, personality disorders, eating disorders, and schizophrenia, as reviewed by Naughton *et al.* (1).

In particular, the serotonin 2 receptor family is associated with mood, anxiety, aggression, and psychophysiological functions such as appetite and sleep (2). The serotonin 2A (5-HT$_{2A}$) receptor exerts its many functions through a wide distribution throughout the cerebral cortex and is also implicated in the action of antidepressant drugs. The role of the 5-HT$_{2A}$ receptor in depression has been extensively studied; the majority of postmortem studies in suicide victims of major depression report increased 5-HT$_{2A}$ binding in the frontal cortex, as reviewed by Arango *et al.* (3). Initial positron emission tomography (PET) and single-photon emission computed tomography studies in depressive patients failed to confirm this finding (4–9), but two recent studies have confirmed the postmortem data. Meyer *et al.* (10) reported that patients with severe depression and high levels of dysfunctional (more pessimistic) attitudes have increased prefrontal cortex 5-HT$_{2A}$ receptor binding. This conclusion was corroborated by Bhagwagar *et al.* (11) that, in addition, showed that recovered, non-medicated patients with a history of recurrent unipolar depression have increased cortical 5-HT$_{2A}$ receptor binding in extensive parts of the cortex.

Many receptor systems in the brain, including the 5-HT$_{2A}$ receptor, exhibit large inter-individual variability in regional receptor binding. Even when the age-related decline in 5-HT$_{2A}$ receptor binding is taken into account, a considerable amount of residual variance remains (12). What causes this variance is not fully understood, but genetic factors are likely to play a role.

The personality dimension neuroticism as assessed from standard personality questionnaires such as the Revised NEO Personality Inventory (NEO-PI-R) (13) and the Eysenck Personality Questionnaire (EPQ) (14) reflects individual differences in the tendency to experience negative emotions such as cheerlessness, sadness, tension, worries, and guilt. A high neuroticism score is found in patients recovered from phobias, panic disorder, and major depression (15), and prospective studies find that a high neuroticism score is associated with an increased risk of developing major depression (16–17). Personality traits show substantial heritability and generally remain stable in adulthood (18). For the neuroticism dimension of the NEO-PI-R the heritability is approximately 40% (19).

On the basis of the evidence mentioned in the preceding text, a relation between neuroticism and serotonergic neurotransmission could possibly exist, and this assumption has been supported by genetic studies. The presence of the functional promotor polymorphism of the serotonin transporter (SERT) gene leading to low expression of SERT enhances the risk of depression, given specific environmental risk factors (20,21), and it is also associated with higher levels of neuroticism, as defined by NEO-PI-R (22,23).

From the Center for Integrated Molecular Brain Imaging and Neurobiology Research Unit (VGF, FAN, SHa, LHP, KHA, CS, SGH, GMK), and PET and Cyclotron Unit (SHo), Copenhagen University Hospital Rigshospitalet; Institute of Public Health (ELM), University of Copenhagen; Danish Research Centre for Magnetic Resonance (OBP), Copenhagen University Hospital, Hvidovre, Copenhagen; Informatics and Mathematical Modelling (FAN), Technical University of Denmark, Lyngby, Denmark.

Address reprint requests to Vibe G. Frokjaer, M.D., Neurobiology Research Unit 9201, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark; E-mail: vibe@nru.dk.

Received April 12, 2007; revised July 4, 2007; accepted July 5, 2007.

0006-3223/08/$34.00
doi:10.1016/j.biopsych.2007.07.009

We hypothesized that neuroticism was associated with frontolimbic 5-HT_{2A} receptor binding, because the frontolimbic regions are involved in both normal and abnormal personality functioning and behavior (24).

Methods and Materials

Subjects

Eighty-three healthy volunteers, 52 men and 31 women, with a mean age of 43 years (range: 18–76), were included. The subjects were recruited through newspaper advertisement. None of the subjects had a history of present or prior neurological or psychiatric disorders, nor did they have first degree relatives with any such disorder. All subjects had a normal neurological examination on the day of PET scanning. None of the subjects took psychoactive drugs. Seven women used hormonal contraception and two used hormonal replacement. Three used non-sedative antihistamine. No subjects used ketanserin, ergots, or ergolines. The studies had been approved by the local ethics committee ([KF] 02-058/99, [KF] 12-122/99, [KF] 12-113/00, [KF] 12-152/01, [KF] 01-001/02, [KF] 11-061/03 and [KF] 12-142/03, [KF] 01-124/04, [KF] 01-156/04). After complete description of the study to the subjects, written informed consent was obtained. Receptor binding data have previously been reported for 49 of the 83 subjects (12).

Imaging

The 5-HT_{2A} receptor binding was imaged with [^{18}F]altanserin PET according to Pinborg et *al.* (25). After bolus-infusion of tracer, emission scans (five frames of 8 min each) are acquired in tracer steady state conditions with an 18-ring GE-Advance scanner (GE, Milwaukee, Wisconsin) operating in three-dimensional–acquisition mode. The total axial field of view was 15.2 cm with an approximate in-plane resolution of 6 mm. After 2 hours, when steady state had been obtained, the fraction of unmetabolized tracer in venous plasma was determined at three time points with high-performance liquid chromatography analysis. Reconstruction, including attenuation correction and scatter correction, is described in detail by Pinborg *et al.* (25). Subjects received a maximum dose of 3.7 MBq/kg bodyweight [^{18}F]altanserin. Structural brain imaging with magnetic resonance (MRI) was conducted in all subjects; magnetization prepared rapid gradient echo (MPRAGE) sequences were acquired on either a 1.5-T Vision scanner (n 68) or a 3-T Trio scanner (both Siemens, Erlangen, Germany) (n 15).

MR/PET Co-Registration

The PET and MR images were co-registered through manual translation and rotation of the PET image with subsequent visual inspection in three planes, with a Matlab-based program (Mathworks, Natick, Massachusetts), as described in Adams *et al.* (12). Data analyses of all PET and MR scans were carried out with the same procedure by the same person.

Volumes of Interest

On the basis of the method by Svarer *et al.* (26), a frontolimbic volume was automatically delineated on each individual's transaxial MRI slices in a strictly user-independent fashion. This method uses a volumes of interest (VOIs) probability map based on a template set of ten MRIs, where VOIs have been defined manually. After alignment, the VOIs were transferred onto the PET images. The frontolimbic region included the orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, anterior cingulate, posterior cingulate, hippocampus, and entorhinal cortex. A volume representing the cerebellum was also defined to assess nonspecific binding.

Quantification of the 5-HT_{2A} Receptor Binding

The outcome parameter was the binding potential of specific tracer binding (BP1). Cerebellum was used as a reference region, because it represents nonspecific binding only. In steady state, BP1 is defined as follows:

$$BP1 = \frac{C_{ROI} - C_{Reference}}{C_{Plasma}} = f_1 \cdot \frac{B_{max}}{K_d} \ (mL\ mL^{-1})$$

where C_{ROI} and $C_{Reference}$ are mean counts in the volume of interest and in the reference region, respectively, C_{Plasma} is the radioactivity originating from parent compound in plasma, f_1 is the free fraction of radiotracer, B_{max} is the density of receptor sites available for tracer binding, and K_d is the affinity constant of the radiotracer to the receptor.

Personality Assessment

Subjects completed the Danish version of the 240-item NEO-PI-R self-report personality questionnaire (27) on the same day as the PET scanning. The NEO-PI-R evaluates the broad personality dimensions of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness. Each dimension or factor score is derived by adding the scores from assessment of six personality traits (facets) and each trait score is derived by adding the scores on eight items in 0–4 Likert format (13). The Danish translation of the NEO-PI-R has been psychometrically evaluated and normed in a standardization sample of 600 subjects. Five of the six traits included in the neuroticism score have high factor loadings on the neuroticism dimension (.67–.84), whereas the loading for impulsiveness is lower (.37) (27). This is in line with the American version of NEO-PI-R (13).

Subjects also completed the Symptom Check-List revised (SCL-90-R) questionnaire (28) on the same day in order to assess symptoms of distress and psychopathology.

Correlation Analysis and Statistics

To test our hypothesis, the neuroticism dimension score was correlated to frontolimbic [^{18}F]altanserin BP1 with adjustment for age and gender in a multiple linear regression analysis with neuroticism as the dependent variable and frontolimbic BP1, age, and gender as independent variables. Parametric statistical methods were chosen, because the neuroticism score and 5-HT_{2A} receptor binding variables passed normality testing (D'Agostino & Pearson omnibus normality test). Age and gender were included as covariates, because neuroticism is known to correlate with gender (27) and the 5-HT_{2A} distribution correlates with age (12). Partial correlation coefficients for the binding are reported. A p value lower than .05 was considered statistically significant. Afterward, to characterize the individual correlations between the six constituent traits of neuroticism and frontolimbic [^{18}F]altanserin BP1, these traits were analyzed as outcome variables. For these tests an uncorrected p value and a p value with Bonferroni correction over the six facets are reported. Also, the uncorrected p value was plotted as a function of the numbers of subjects included based on 100 resamplings of each n (1–83) from the total cohort (n 83).

Voxel-Based Analysis

The PET images were transformed and warped into a common brain atlas (Montreal Neurological Institute [MNI]-space) by means of statistical parametric mapping (SPM2, Wellcome Department of Imaging Neuroscience, London, United Kingdom). The spatial normalization algorithm in SPM2 estimated a warp-field from the individual MPRAGE MRI to the MNI152 T1

Table 1. Correlation Between Neuroticism and Constituent Trait Scores and Frontolimbic 5-HT_{2A} Binding

Neuroticism	*r* .24, *p*	.028
Vulnerability	*r* .35, *p*	.0015
Anxiety	*r* .24, *p*	.032
Depression	*r* .21, *p*	.063
Self-Consciousness	*r* .18, *p*	.11
Impulsiveness	*r* .01, *p*	.95
Angry Hostility	*r* .06, *p*	.56

r partial correlations from multiple linear regression analysis with the personality score as the dependent variable and frontolimbic serotonin 2A (5-HT_{2A}) receptor binding potential, age, and gender as covariates. *p* values are uncorrected.

template provided in SPM99 (29). We applied these warp-fields to the co-registered PET and MRI scans, resulting in volumes in MNI-space with 2 mm isotropic voxels. After spatial normalization, each PET-volume was spatially smoothed with a Gaussian kernel with 12 mm full width half maximum.

Parametric BP1 images were determined from the warped and smoothed PET-volumes after subtraction of the nonspecific binding value of the cerebellum ($C_{reference}$), derived from the regional analysis, and division by the plasma concentration of unmetabolized tracer (C_{plasma}). The voxel-wise multiple regression analyses paralleled those for the analysis of the regional BP1 data, as described earlier, with thresholds of voxel-level, uncorrected, two-tailed p .05, p .01, and p .001, respectively, as illustrated in Figure 4. Only voxels within the brain were tested, and coordinates representing local maxima in the volume of partial correlation coefficients were extracted. Anatomical labeling of these coordinates was performed by examining the position of the coordinates on the mean image of the warped MRIs of all subjects supported by the labels from the AAL (Anatomical Automatic Labelling) volume (30). All significant clusters, at the voxel-level, two-tailed, uncorrected, p .001, are reported in Tables 2 and 3. Only clusters larger than 10 voxels are reported. Only coordinates of maxima within each cluster further apart than 8 mm are listed. Tmax value and the corresponding p value are given for the most significant maximum in the cluster.

Results

Effects of Age, Gender, and Body Mass Index

As previously demonstrated (12) there was no main effect of gender on 5-HT_{2A} receptor binding, whereas the 5-HT_{2A} receptor binding was negatively correlated to age in all cortical regions (p .000001, r varying between .51 and .78, frontolimbic r .62). Neuroticism score was not dependent on age but tended to be slightly higher for women than men, r .20, p .069, as expected (27). Because previous data have suggested an interaction between body mass index (BMI) and 5-HT_{2A} receptor binding (12), we tested whether the addition of BMI as a covariate changed the correlation between neuroticism or constituent traits and 5-HT_{2A} receptor binding. This was not the case; consequently, BMI was left out of the analysis.

Predicting Neuroticism from Frontolimbic 5-HT_{2A} Receptor Binding

As hypothesized, the neuroticism score correlated positively with frontolimbic 5-HT_{2A} receptor binding [r(79) .24, p .028] (Figure 1). Post hoc analysis of the constituent traits of neuroticism showed that the correlation was strongest for vulnerability [r(79) .35, p .0015, uncorrected] (Figure 2) and anxiety [r(79) .24, p .032, uncorrected] (Table 1). The positive correlation between frontolimbic 5-HT_{2A} receptor binding and vulnerability survived a conservative correction for multiple comparisons (p .009, Bonferroni corrected). As evident from Figure 3, the constituent traits of neuroticism seemed to exhibit different relationships to 5-HT_{2A} receptor binding, with vulnerability exhibiting a strong association with binding; anxiety, depression, and self-consciousness: intermediate; and impulsiveness and angry hostility: little or no association with binding.

The positive correlation found in the region-based analysis was confirmed and further characterized in a voxel-based analysis. Figure 4 illustrates that the positive correlations between 5-HT_{2A} receptor binding and neuroticism or vulnerability were present primarily in frontal, temporal, and left insular cortex, and in the posterior cingulate. Tables 2 and 3 report the anatomical localization, cluster size, Tmax, and voxel level p value for each significant cluster, filtered at voxel-level, uncorrected, two-tailed p .001.

A post hoc region-based analysis was conducted to explore correlations between 5-HT_{2A} receptor binding and neuroticism both in brain regions outside the frontolimbic region and within the frontolimbic subregions. Within the frontolimbic region, the most pronounced correlations were found in entorhinal cortex, superior frontal cortex, posterior cingulate, and inferior frontal cortex, as illustrated in Table 4. Outside the frontolimbic region a positive correlation was also present in insula (r .22, p .044, uncorrected) and superior temporal cortex (r .23, p .040, uncorrected). Neuroticism did not correlate to 5-HT_{2A} receptor binding in any other cortical region, midbrain, or white matter (data not shown). There was no significant correlation between f1 or nonspecific radiotracer binding and neuroticism or any of its constituent trait scores.

Personality Profile of the Study Sample

The personality profile of the study sample deviated only marginally from the profile of 600 healthy Danish subjects (27). Only for the dimension openness was a significantly higher score found in our sample (114 19, vs. 104 20, mean SD, p .0016), probably reflecting that our subjects all volunteered for a PET-study. The neuroticism, vulnerability, anxiety, and depression mean scores of our sample were comparable to that of the

Table 2. Voxel-Based Analysis of the Correlation Between Neuroticism and 5-HT_{2A} Receptor Binding

#	Anatomical Label (x, y, z Coordinates)	Cluster Size (Voxels)	Tmax-Value	*p*
1	Left Insula (28, 32, 6)	123	4.01	.00014
2	Right Parahippocampal Gyrus (30, 22, 28)	80	3.89	.00020

Anatomical label with coordinates of maxima within the cluster, further than 8 mm apart, in brackets. Tmax value and corresponding *p* value is given for the most significant maximum. Threshold: p .001, voxel-level, uncorrected, two-tailed *p* values, cluster size 10 voxels. Voxel size 2 2 2 mm, (i.e., 1 cm^3 corresponds to 125 voxels).

5-HT_{2A}, serotonin 2A; #, cluster number.

Table 3. Voxel-Based Analysis of the Correlation Between Vulnerability and 5-HT_{2A} Receptor Binding

#	Anatomical Label (x, y, z Coordinates)	Cluster Size (Voxels)	Tmax Value	*p*
1	Left insula and superior, middle, and inferior frontal gyrus: (28,34,4), (26,60,22), (2,52,44), (48,46,0), (14,44,50), (4,66,12), (4,62,26), (40,8,60), (30,40, 16). Right superior frontal gyrus, orbital, and dorsolateral part: (2,62, 14), (16,56,36).	4226	5.05	2.7E-06
2	Left superior temporal gyrus: (66, 22,18), (62, 2,20), (58,10, 2), (66, 48,16), (46,2, 14), (64, 8,2), (60, 26,42)	2402	4.54	1.9E-05
3	Right inferior, orbital, and middle frontal gyrus: (46,52,12), (50,46, 2), (54,32,24), (30,62, 6)	1445	4.56	1.8E-05
4	Left occipital and posterior cingulate: (22, 62,36), (12, 48,24)	712	4.19	7.3E-05
5	Right fusiform gyrus (32, 18, 30)	570	4.70	1.1E-05
6	Right precuneus (24, 56,30), (16, 52,26)	257	3.86	.00023
7	Right superior frontal gyrus; dorsolateral: (36, 4,64), (22,24,62)	249	3.81	.00027
8	Right superior temporal pole: (60,8, 4), (64,0,10)	247	3.97	.00016
9	Left fusiform gyrus (30, 20, 32)	212	3.88	.00021
10	Right superior temporal gyrus (68, 28,12)	54	3.68	.00042
11	Left middle temporal pole (36,22, 32)	13	3.65	.00047
12	Right middle temporal gyrus (66, 50, 4)	12	3.48	.00081
13	Left middle temporal gyrus (44, 48,8)	12	3.52	.00073

Anatomical label with coordinates of maxima within the cluster, further than 8 mm apart, in brackets. Tmax value and corresponding *p* value is given for the most significant maximum. Threshold: *p* .001, voxel-level, uncorrected, two-tailed *p* values, cluster size 10 voxels. Voxel size 2 2 2 mm (i.e., 1 cm^3 corresponds to 125 voxels).

5-HT_{2A}, serotonin 2A; #, cluster number.

Danish standardization sample. The results from a simultaneously conducted SCL-90-R questionnaire assessment (28) confirmed the absence of psychopathology, in particular depression, thus confirming that our sample reflects variations within the normal range.

Discussion

This study is the largest receptor imaging study reported to date. In 83 healthy subjects we found a significant positive correlation between neuroticism and frontolimbic 5-HT_{2A} receptor binding [$r(79)$ .24, p .028] that seemed to be strongest for two constituent traits of neuroticism, vulnerability and anxiety. Indeed, vulnerability displayed the most pronounced correlation (r .35, p .009, Bonferroni corrected), and the findings were confirmed in a voxel-based analysis. We first identified the correlation in the sample of 49 subjects included in Adams *et al.* (12) but did not report the observation at that time, because we wanted to confirm it in a larger sample. After inclusion of an additional 34 subjects, we find that the correlation remains statistically significant.

In a sample of five postmenopausal women, estradiol and progesterone administration has been reported to be associated with an increased cortical 5-HT_{2A} receptor binding (31). By contrast, we observed neither a gender effect on 5-HT_{2A} receptor binding nor an effect of hormonal contraception or replacement therapy within the female group (n 31). Therefore, in our sample endogenous or therapeutic levels of estradiol did not significantly affect 5-HT_{2A} receptor binding. In addition, the

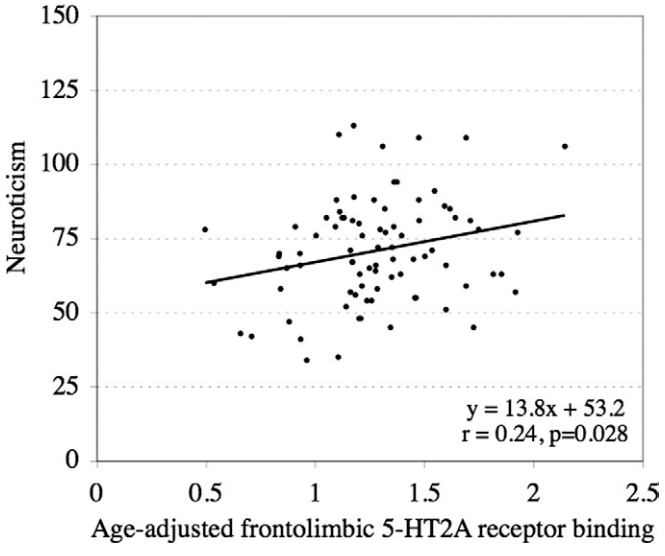


Figure 1. The correlation between neuroticism and age-adjusted frontolimbic serotonin 2A (5-HT_{2A}) receptor binding. When adjusting, the slope of the line for the age predictor was used to calculate each subject's frontolimbic 5-HT_{2A} receptor binding to that expected for a 40-year-old subject.

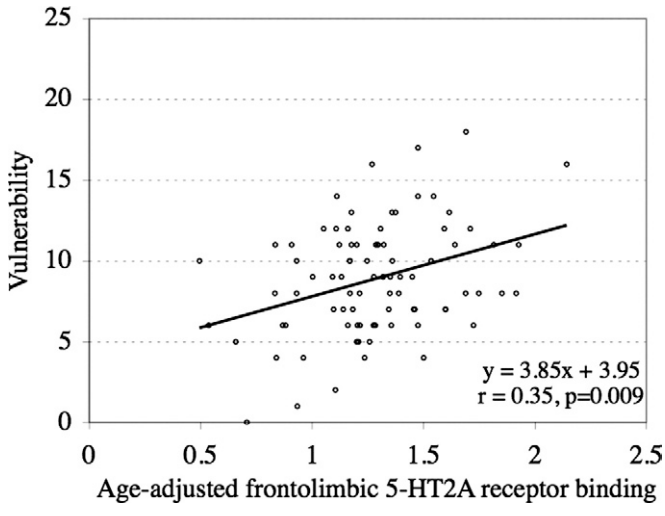


Figure 2. The correlation between vulnerability and age-adjusted frontolimbic serotonin 2A (5-HT_{2A}) receptor binding. When adjusting, the slope of the line for the age predictor was used to calculate each subject's frontolimbic 5-HT_{2A} receptor binding to that expected for a 40-year-old subject.

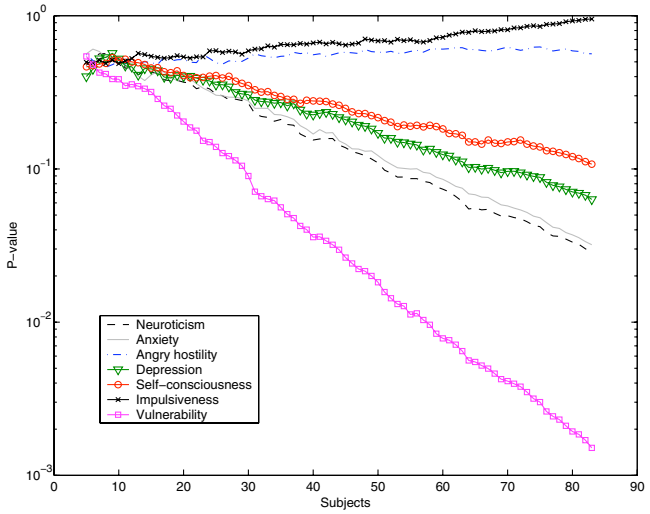


Figure 3. The correlations between the six constituent facets of neuroticism and frontolimbic serotonin 2A (5-HT_{2A}) receptor binding as compared with the correlation with neuroticism itself as a function of number of included subjects. The calculated *p* value for the positive correlation between each facet score and the frontolimbic 5-HT_{2A} receptor binding for each number of subjects included (*n*: 1–83) is based on random resampling 100 times in the total cohort.

correlation between neuroticism and vulnerability and 5-HT_{2A} receptor binding was also present in the group of men only, *n* 52 (neuroticism: *r* .28, *p* .049; vulnerability: *r* .37, *p* .007, uncorrected).

The partial correlations were modest, corresponding to an explained variance of up to 12% for vulnerability. It should, however, be born in mind that personality is a highly complex phenomenon influenced by a multitude of factors and, consequently, correlations between 5-HT_{2A} receptor binding in individual brain regions and complex psychological traits might only be detectable in a relatively large sample, such as ours. In addition, correlations depend on the sample variance, which will tend to be moderate in a sample of healthy subjects without documented personality disorders. However, the aim of this study was to investigate a mentally healthy sample to avoid bias from significant life events such as depression or treatment effects.

Table 4. Correlation Between Neuroticism and 5-HT_{2A} Binding in Frontolimbic Regions

	Neuroticism	
Orbitofrontal Cortex	*r* .21, *p*	.059
Medial Inferior Frontal Cortex	*r* .24, *p*	.034
Superior Frontal Cortex	*r* .26, *p*	.020
Anterior Cingulate Cortex	*r* .18, *p*	.11
Posterior Cingulate Cortex	*r* .24, *p*	.031
Entorhinal Cortex	*r* .30, *p*	.006
Hippocampus	*r* .20, *p*	.073

p uncorrected *p* value, and *r* partial correlations from multiple linear regression analysis with neuroticism as the dependent variable and regional serotonin 2A (5-HT_{2A}) receptor binding potential, age, and gender as covariates.

This is the first study to examine the association between neuroticism and selective 5-HT_{2A} receptor binding in the human brain. A small-scale PET study (32) including 11 healthy subjects has previously reported a negative correlation between cortical binding of the non-selective 5-HT_2 tracer [^{18}F]-fluoroethyl-spiperone and the personality trait harm avoidance, as measured by the Tridimensional Personality Questionnaire (TPQ) of Cloninger *et al.* (33). Because TPQ does not offer a direct measure of neuroticism and we used a selective 5-HT_{2A} PET tracer, the two studies are not directly comparable.

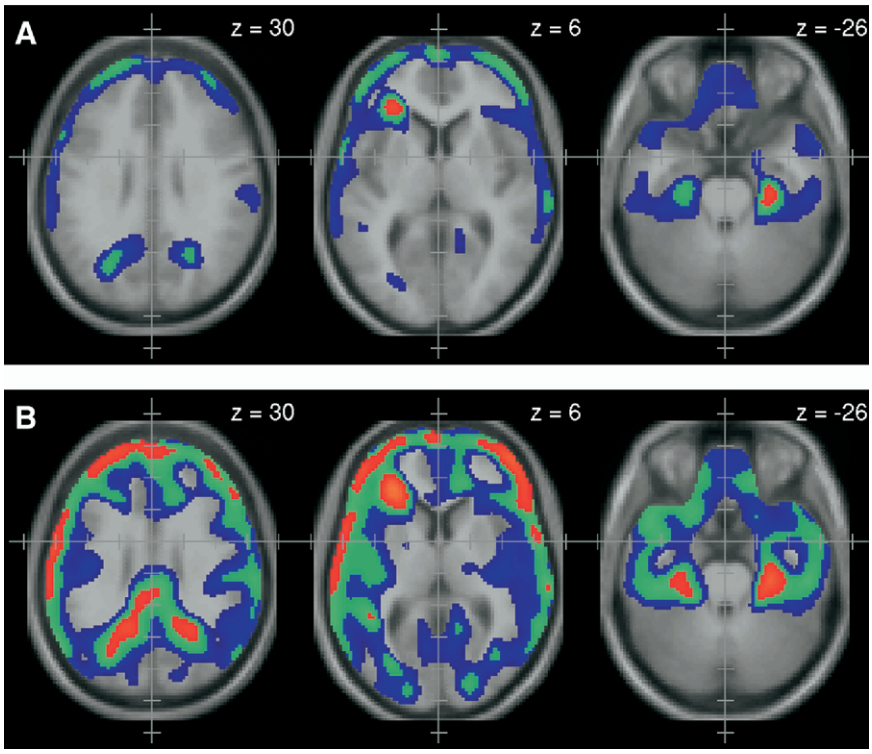


Figure 4. Positive correlations between neuroticism (upper panel) and vulnerability (lower panel) and serotonin 2A (5-HT_{2A}) receptor binding from voxel-based analysis of BP1 parametric images. The color scale refers to different significance levels: blue: *p* .05; green: *p* .01; red: *p* .001; voxel-level, uncorrected, two-tailed *p*. The probability maps are projected onto a mean magnetic resonance image derived from the 83 subjects after transformation to Montreal Neurological Institute (MNI) space.

Neuroticism, Constitutive Traits, and Depressive Disorder

Neuroticism has consistently been found to be associated with the risk of developing depression (16,17), but so far, no studies have attempted to define which constitutive traits are more important in this respect. Vulnerability showed the strongest correlations with frontolimbic 5-HT_{2A} receptor binding, suggesting that factors assessed with this scale might be particularly important mediators of the association between neuroticism and 5-HT_{2A} receptor binding. Because there is a high degree of correlation between neuroticism and its constitutive trait vulnerability (r .75–.79) (27), we assume that a high vulnerability score is also associated with an increased risk of developing depression. Vulnerability is based on self-reported answers to eight questions exploring the subject's ability to cope with stress and critical situations, general need for help and support, decisiveness, doubting, and emotional stability (13). That is, the vulnerability score particularly assesses stress-coping strategies, which might explain why we find the most pronounced correlations between 5-HT_{2A} receptor binding and the trait vulnerability as a reflection of the known associations between stress and risk of developing a depression (20,21,34). It would have been interesting to investigate the potential role of additional markers, such as cortisol, on the response to emotional stress. Because these data were not available, unfortunately, we cannot elaborate on the potential relation between vulnerability score and physiological stress response in this sample.

The majority of postmortem studies of depressive suicide victims report increased 5-HT_{2A} binding in prefrontal and frontal cortices (3), and one recent PET study has found increased cortical 5-HT_{2A} receptor binding in euthymic, medication-free patients recovered from depression as compared with healthy subjects (11). Another PET-study reports an increase in cortical, particularly prefrontal 5-HT_{2A} binding in patients with severe major depressive disorder and high levels of dysfunctional attitudes (10). In depressed patients, Mintun *et al.* (9) previously reported an isolated decrease in hippocampal 5-HT_{2A} receptor binding but no significant differences in cortical regions. More recent data have not been able to replicate these initial findings. Although the study of Mintun *et al.* included a large number of patients, a power analysis of [^{18}F]altanserin-PET data (35) has shown that to avoid type II errors, robust detection of differences in hippocampus requires a sample size twice the order of his sample. Thus, our findings in healthy subjects are in accordance with findings in depressive subjects as evaluated from postmortem studies as well as two recent imaging studies on cortical in vivo 5-HT_{2A} receptor binding, suggesting that high frontolimbic 5-HT_{2A} receptor binding could be a trait characteristic for proneness to develop depression.

Anxiety tended to correlate positively with frontolimbic 5-HT_{2A} binding (p .032, uncorrected). Whether serotonergic neurotransmission is implicated specifically in anxiety in healthy subjects is not clear, and previous findings are contradictory. Whereas Tauscher *et al.* (36) observed a negative correlation between serotonin 1A binding and the NEO-PI-R anxiety trait in healthy subjects (n 19), a larger study involving 44 male volunteers by Rabiner *et al.* (37) failed to identify any significant correlations between EPQ-assessed neuroticism and the serotonin 1A receptor binding. Interestingly, Weisstaub *et al.* (38) found that mice genetically modified to lack 5-HT_{2A} receptor signalling were less inhibited by potential risk (i.e., they were more likely to choose to explore new areas or feed in unknown environments despite a potential risk). This study suggests that high cortical 5-HT_{2A} signalling co-exists with a high tendency to perceive or judge an environment as risky.

Although the traits vulnerability and anxiety both reflect sensitivity to stress and trauma, two other facets of neuroticism, angry hostility and impulsiveness, clearly reflect a disposition to more active reaction when faced with frustration. Interestingly, the angry hostility and impulsiveness facets of neuroticism did not seem to contribute to the correlation between neuroticism and high 5-HT_{2A} receptor binding, as illustrated in Figure 3. To our knowledge, there are no published data available to determine whether high angry hostility or impulsiveness significantly contributes to the risk of developing depressive disorder; our data are suggestive of the contrary.

In support of a neurobiological link between the serotonergic system and neuroticism, other key-elements in the serotonergic neurotransmission also show associations to neuroticism. The SERT-binding was positively correlated to neuroticism in a sample of 31 healthy male volunteers (39) and to negativistic thinking in depression (40). Whether the positive correlation between neuroticism and both SERT and 5-HT_{2A} is driven by a common factor (i.e., genetic or a developmental effect) is unknown. Future combined PET studies on pre- and postsynaptic serotonin receptor binding in the same subjects will be needed to study the dynamics between SERT and 5-HT_{2A} in vivo and possible additive roles in the relation to neuroticism or risk of developing depression.

In healthy subjects a high neuroticism score is associated with low levels of serum brain derived neurotrophic factor (BDNF) (41) and high waking salivary cortisol levels (42). Both low serum BDNF and high levels of cortisol are observed in subjects with major depression (43,44) and are considered risk factors for development of depression (41,45,46) in parallel with high neuroticism scores (16,17). Our finding of increased 5-HT_{2A} receptor binding is well in line with the observation that central 5-HT_{2A} agonism stimulates cortisol excretion (47), and it might also reflect the observation from animal studies that enhanced cortical 5-HT_{2A} signalling is accompanied by a tendency to perceive or judge an environment as risky (38). In contrast, an upregulation of 5-HT_{2A} receptor levels in response to low serotonin levels, possibly associated with neuroticism, might also play a role (2,48). The explanation of possible mechanisms remains speculative, because synaptic levels of serotonin cannot be measured in vivo. However, the correlation between 5-HT_{2A} receptor binding and neuroticism might also reflect serotonergic modulations in early brain development. Several studies point toward a critical role of serotonin in the maturation of brain systems that modulate emotional function in the adult (49).

An explanation for the association between high neuroticism and increased risk of developing depression is offered by Fanous *et al.* (50), who showed that the relationship between neuroticism and major depression to a large extent is due to common genetic factors predisposing to both neuroticism and major depression. Such factors are likely to be polygenic, modulated by specific environmental factors, and not restricted to single gene effects, including polymorphisms of the 5-HT_{2A} receptor gene. Indeed, several large studies (N 239, N 177, N 133) have not identified associations between 5-HT_{2A} receptor gene polymorphisms and neuroticism (51–53) or depression (54). However, personality also shapes the response to environmental risk factors such as stressful life events and might interact in the relationship between genetic vulnerability and environmental risk factors (55). We speculate that the high frontolimbic 5-HT_{2A} binding in healthy subjects with high neuroticism scores could be

a genetically determined trait that might lead to a depressive state when a subject is exposed to stressful stimuli. Future studies must elucidate whether genetic predisposition to depression in subjects without depressive symptoms is related to specific changes in cortical 5-HT_{2A} receptor binding.

Conclusions

In this large sample of healthy subjects we found that the neuroticism score was positively correlated with frontolimbic 5-HT_{2A} receptor binding. This correlation was particularly strong for the constituent trait of neuroticism, vulnerability, defined as a person's difficulties in coping with stress. Whether 5-HT_{2A} binding influences the development of specific personality traits or whether the observed correlations reflect common genetic or environmental factors remains to be determined. Higher 5-HT_{2A} receptor binding in nondepressed people with a high vulnerability score might reflect a neural substrate, possibly genetically determined, of risk to develop depression. This notion is supported by the reports on higher 5-HT_{2A} receptor binding in patients recovered from depression. Our findings offer a link between personality within the normal range and affective disorders and suggest that in the normal brain serotonergic neurotransmission is associated with individual differences in neuroticism and, in particular, its constituent personality trait vulnerability.

This work was generously supported by Danish Medical Research Council, The Health Science Faculty, University of Copenhagen, the 1991 Pharmacy Foundation, The Lundbeck Foundation, the EU Fifth framework programme, Sawmill owner Jeppe Juhl and Wife Ovita Juhls Foundation, "Laegernes forsikringsforening af 1891," and "Fonden af 1870."

We wish to thank Karin Stahr, Dorthe Givard, and the staff at the PET Centre, Rigshospitalet, for their technical assistance. Anders Gade is thanked for helpful suggestions in designing the study. Terry Jernigan is thanked for helpful discussions, most valuable critique, and participation in preparing the manuscript. The John and Birthe Meyer Foundation is thanked for the donation of the Cyclotron and PET scanner.

The authors declare no competing financial interest.

1. Naughton M, Mulrooney JB, Leonard BE (2000): A review of the role of serotonin receptors in psychiatric disorders. *Hum Psychopharmacol* 15: 397–415.
2. Roth BL, Berry SA, Kroeze WK, Willins DL, Kristiansen K (1998): Serotonin 5-HT2A receptors: Molecular biology and mechanisms of regulation. *Crit Rev Neurobiol* 12:319–338.
3. Arango V, Underwood MD, Mann JJ (1997): Postmortem findings in suicide victims. Implications for in vivo imaging studies. *Ann N Y Acad Sci* 836:269–287.
4. Attar-Levy D, Martinot JL, Blin J, Dao-Castellana MH, Crouzel C, Mazoyer B, *et al.* (1999): The cortical serotonin2 receptors studied with positron-emission tomography and [18F]-setoperone during depressive illness and antidepressant treatment with clomipramine. *Biol Psychiatry* 45: 180–186.
5. Meyer JH, Kapur S, Houle S, DaSilva J, Owczarek B, Brown GM, *et al.* (1999): Prefrontal cortex 5-HT2 receptors in depression: An [18F]setoperone PET imaging study. *Am J Psychiatry* 156:1029–1034.
6. Yatham LN, Liddle PF, Shiah IS, Scarrow G, Lam RW, Adam MJ, *et al.* (2000): Brain serotonin 2 receptors in major depression: A positron emission tomography study. *Arch Gen Psychiatry* 57:850–858.
7. van Heeringen C, Audenaert K, Van Laere K, Dumont F, Slegers G, Mertens J, *et al.* (2003): Prefrontal 5-HT2A receptor binding index, hopelessness and personality characteristics in attempted suicide. *J Affect Disord* 149–158.
8. Messa C, Colombo C, Moresco RM, Gobbo C, Galli L, Lucignani G, *et al.* (2003): 5-HT(2A) receptor binding is reduced in drug-naive and unchanged in SSRI-responder depressed patients compared to healthy controls: A PET study. *Psychopharmacology* 167:72–78.
9. Mintun MA, Sheline YI, Moerlein SM, Vlassenko AG, Huang Y, Snyder AZ, *et al.* (2004): Decreased hippocampal 5-HT2A receptor binding in major depressive disorder: In vivo measurement with [18F]Altanserin positron emission tomography. *Biol Psychiatry* 55:217–224.
10. Meyer JH, McMain S, Kennedy SH, Korman L, Brown GM, DaSilva JN, *et al.* (2003): Dysfunctional attitudes and 5-HT2 receptors during depression and self harm. *Am J Psychiatry* 160:90–99.
11. Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P (2006): Increased 5-HT2A receptor binding in euthymic, medication-free patients recovered from depression: A positron emission study with [11C]MDL 100,907. *Am J Psychiatry* 163:1580–1587.
12. Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S, *et al.* (2004): A database of [18F]-altanserin binding to 5-HT2A receptors in normal volunteers: Normative data and relationship to physiological and demographic variables. *NeuroImage* 21:1105–1113.
13. Costa PT, McCrae RR (1992): *Professional Manual for Revised NEO Personality Inventory*. Odessa, Florida: Psychological Assessment Resources.
14. Eysenck HJ, Eysenck SBG (1991): *Manual of the Eysenck Personality Scales (EPS Adult)*. London & Sydney: Hodder & Stoughton.
15. Bienvenu OJ, Nestadt G, Samuels JF, Costa PT, Howard WT, Eaton WW (2001): Phobic, panic and major depressive disorders and the five-factor model of personality. *J Nerv Ment Dis* 189:154–161.
16. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ (1993): A longitudinal twin study of personality and major depression in women. *Arch Gen Psychiatry* 50:853–862.
17. Boyce P, Parker G, Barnett B, Cooney M, Smith F (1991): Personality as a vulnerability factor to depression. *Br J Psychiatry* 159:106–114.
18. Caspi A, Roberts BW, Shiner RL (2005): Personality development: Stability and change. *Annu Rev Psychol* 56:453–484.
19. Jang KJ, Livesley WJ (1996): Heritability of the big five personality dimensions and their facets: A twin study. *J Pers* 64:577–591.
20. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, *et al.* (2003): Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science* 301:386–389.
21. Kendler SK, Kuhn JW, Vittun J, Prescott CA, Riley B (2005): The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression. *Arch Gen Psychiatry* 62:529–535.
22. Greenberg BD, Li Q, Lucas FR, Hu S, Sirota LA, Benjamin J, *et al.* (2000): Association between the serotonin transporter promotor polymorphism and personality traits in a primarily female population sample. *Am J Med Genet* 96:202–216.
23. Schinka JA, Busch RM, Robichaux-Keene N (2004): A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. *Mol Psychiatry* 2:197–202.
24. Bradshaw JL (2001): *Developmental Disorders of the Frontostriatal System*. East Sussex, United Kingdom: Psychology Press.
25. Pinborg LH, Adams KH, Svarer C, Holm S, Hasselbalch SG, Haugbol S, *et al.* (2003): Quantification of 5-HT2A receptors in the human brain using [18F]altanserin-PET and the bolus/infusion approach. *J Cereb Blood Flow Metab* 23:985–96.
26. Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, *et al.* (2005): MR-based automatic delineation of volumes of interest in human brain PET-images using probability maps. *Neuroimage* 24:969–979.
27. Hansen HS, Mortensen EL, Schiøtz HK (2004): NEO-PI-R, manual—klinisk. In: Hansen HS, Mortensen EL, editors. *Dokumentation for den danske udgave af NEO PI-R og NEO PI-R Kort Version*. Copenhagen, Denmark: Dansk Psykologisk Forlag.
28. Derogatis LR (1994): *SCL-90-R: Symptom Checklist-90-R. Administration, Scoring and Procedures Manual, 3rd ed*. Minneapolis, Minnesota: National Computer Systems.
29. Talairach J, Tournoux P (1988): *Co-Planar Stereotaxic Atlas of the Human Brain*. New York: Thieme Medical Publisher.
30. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, *et al.* (2002): Automated anatomical labelling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15:273–289.

31. Moses EL, Drevets WC, Smith G, Mathis CA, Kalro BN, Butters MA, *et al.* (2000): Effects of estradiol and progesterone administration on human serotonin 2A receptor binding: A PET study. *Biol Psychiatry* 48:854–860.
32. Moresco FM, Dieci M, Vita A, Messa C, Gobbo C, Galli L, *et al.* (2002): In vivo serotonin 5HT(2A) receptor binding and personality traits in healthy subjects: A positron emission tomography study. *NeuroImage* 17:1470–1478.
33. Cloninger CR, Przybeck TR, Svakic DM, Wetzel RD (1994): *The Temperament and Character Inventory (TCI): A Guide to Its Development and Use*. Washington DC: Washington University, Center for Psychobiology of Personality.
34. Kendler KS, Kuhn J, Prescott CA (2004): The interrelationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression. *Am J Psychiatry* 161:631–636.
35. Haugbol S, Pinborg LH, Arfan HM, Frokjaer VM, Madsen J, Dyrby TB, *et al.* (2007): Reproducibility of 5-HT(2A) receptor measurements and sample size estimations with [(18)F]altanserin PET using a bolus/infusion approach. *Eur J Nucl Med Mol Imaging* 34:910–915.
36. Tauscher J, Bagby RM, Javanmard M, Christensen BK, Kasper S, Kapur S (2001): Inverse relationship between serotonin 5-HT1A receptor binding and anxiety: A [11C]WAY-100635 PET investigation in healthy volunteers. *Am J Psychiatry* 158:1326–1328.
37. Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD, *et al.* (2002): A database of [(11)C]WAY-100635 binding to 5-HT(1A) receptors in normal male volunteers: Normative data and relationship to methodological, demographic, physiological, and behavioral variables. *Neuroimage* 15:620–632.
38. Weisstaub NV, Zhou M, Lira A, Lambe E, González-Maeso J, Hornung JP, *et al.* (2006): Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. *Science* 313:536–540.
39. Takano A, Arakawa R, Hayashi M, Takahashi H, Ito H, Suhara T (2007): Relationship Between Neuroticism Personality Trait and Serotonin Transporter Binding. *Biol Psychiatry* March 2, 2007 [Epub ahead of print].
40. Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N, *et al.* (2004): Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: Effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry* 61:1271–1279.
41. Lang UE, Hellweg R, Gallinat J (2004): BDNF serum concentrations in healthy volunteers are associated with depression-related personality traits. *Neuropsychopharmacology* 29:795–798.
42. Portella MJ, Harmer CJ, Flint J, Cowen P, Goodwin GM (2005): Enhanced early morning salivary cortisol in neuroticism. *Am J Psychiatry* 162:807–809.
43. Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM (2002): Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res* 109:143–148.
44. Holsboer F (2001): Stress, hypercortisolism and corticosteroid receptors in depression: Implications for therapy. *J Affect Disord* 62:77–91.
45. Modell S, Huber J, Holsboer F, Lauer CJ (2003): The Munich Vulnerability Study on Affective Disorders: Risk factors for unipolarity versus bipolarity. *J Affect Disord* 74:173–184.
46. Mannie ZN, Harmer CJ, Cowen PJ (2007): Increased waking salivary cortisol levels in young people at familial risk of depression. *Am J Psychiatry* 2007;164:617–621.
47. Van de Kar LD, Javed A, Zhang Y, Serres F, Raap DK, Gray TS (2001): 5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate hypothalamic CRF and oxytocin-expressing cells. *J Neurosci* 2001;21:3572–3579.
48. Stockmeier C, Kellar K (1986): In vivo regulation of the serotonin-2 receptor in rat brain. *Life Sci* 38:117–127.
49. Gaspar P, Cases O, Maroteaux L (2003): The developmental role of serotonin: News from mouse molecular genetics. *Nat Rev Neurosci* 4:1002–1012.
50. Fanous A, Gardner CO, Prescott CA, Cancro R, Kendler KS (2002): Neuroticism, major depression and gender: A population-based twin study. *Psychol Med* 32:719–728.
51. Tochigi M, Umekage T, Kato C, Marui T, Otowa T, Hibino H, *et al.* (2005): Serotonin 2A receptor gene polymorphism and personality traits: No evidence for significant association. *Psychiatr Genet* 15:67–69.
52. Jonsson EG, Nothen MM, Gustavsson JP, Berggard C, Bunzel R, Forslund K, *et al.* (2001): No association between serotonin 2A receptor gene variants and personality traits. *Psychiatr Genet* 11:11–17.
53. Kusumi I, Suzuki K, Sasaki Y, Kameda K, Sasaki T, Koyama T (2002): Serotonin 5-HT(2A) receptor gene polymorphism, 5-HT(2A) receptor function and personality traits in healthy subjects: A negative study. *J Affect Disord* 68:235–241.
54. Oswald P, Souery D, Massat I, Del-Favero J, Linotte S, Papadimitriou G, *et al.* (2003): Lack of association between the 5HT2A receptor polymorphism (T102C) and unipolar affective disorder in a multicentric European study. *Eur Neuropsychopharmacol* 13:365–368.
55. Jacobs N, Kenis G, Peeters F, Derom C, Vlietinck R, van Os J (2006): Stress-related negative affectivity and genetically altered serotonin transporter function. *Arch Gen Psychiatry* 63:989–996.

Paper 2

Frontolimbic serotonin 2A receptor binding, neuroticism, and familial risk for mood disorder

Vibe G. Frokjaer[1], Maj Vinberg[2], Wiliam Baare[4], David Erritzoe[1], Klaus Kähler Holst[5], Erik Lykke Mortensen[6], Haroon Arfan[1], Jacob Madsen[3], Lars V. Kessing[2], Gitte M. Knudsen[1]

[1]Neurobiology Research Unit and Center for Integrated Molecular Brain Imaging, [2]Department of Psychiatry, and [3]PET and Cyclotron Unit Copenhagen University Hospital Rigshospitalet, [4]Danish Research Centre for Magnetic Resonance, Hvidovre University Hospital, [5]Department of Biostatistics, and [6]Institute of Public Health, University of Copenhagen, Denmark.

Corresponding author:
Vibe G. Frokjaer, M.D.
Neurobiology Research Unit 9201
Copenhagen University Hospital, Rigshospitalet
Blegdamsvej 9, DK-2100 Copenhagen, Denmark
Telephone: +45 35456712, Fax: +45 356713.
Email: vibe@nru.dk

Abstract

Mood disorders are elicited by a combination of genetic and environmental stress factors. We have previously demonstrated that in healthy subjects a certain personality trait, neuroticism, known to be associated with increased risk of developing depression, is positively correlated to frontolimbic serotonin 2A receptor binding. Here we tested the hypothesis that healthy twins at high risk for developing mood disorder, have a pronounced positive coupling between frontolimbic serotonin 2A receptor binding and neuroticism.

Twenty-one healthy twins with a co-twin history of mood disorder and 17 healthy, low-risk twins with a co-twin without a history of mood disorder were included. They answered self-report personality questionnaires and were investigated with [18F]-altanserin positron emission tomography (PET) and magnetic resonance imaging (MRI).

In an analysis adjusting for age and body mass index we found that within the high-risk group, neuroticism and frontolimbic serotonin 2A receptor binding was positively associated ($p=0.0049$). The association was not found in the low-risk group ($p=0.25$), and the difference between the groups was statistically significant ($p=0.030$).

In conclusion, a complex association between familial risk for mood disorders, frontolimbic serotonin 2A receptor binding, and neuroticism exists. We speculate that the combination of high frontolimbic serotonin 2A receptor binding and high neuroticism might be particularly disadvantageous for individuals at high risk for mood disorders, by enhancing the negative impact of stress.

Introduction

The most potent risk factor for mood disorder is a family history of the disease. In particular, recurrence and early age of onset are characteristic for cases with the greatest familial risk (*1-3*). It is most likely a combination of genetic predisposition and environmental stress factors that elicits the mood disorder. For example, Caspi et al (*4*) showed how the low expressing variant of the polymorphism in the promoter region of the serotonin transporter gene and life stress interact to develop major depression.

Twin studies have shown that the heritability of depression is approximately 40% (*2, 5-7*) that the heritability of depression is higher in women than in men, and that some genetic risk factors are gender-related (*7*). The observation that depression is twice as frequent in women may be explained by to gender-specific differences in genetic vulnerability to environmental stress (*8, 9*). Although modest single gene effects have been identified, the heritability is predominantly polygenic (*10*). Recent studies of the genetic background for depression focus on two phenotypes, the occurrence of major depression itself and the personality trait neuroticism, a robust predictor of future onset of major depression (*11, 12*). Some of the genetic factors influencing the development of the personality trait neuroticism may also increase the risk of major depression. In a large twin population of 20.692 members of same-sex twin pairs the genetic correlation between neuroticism and depression was estimated to be +0.46 (*13*); this correlation does not seem to be gender specific (*14*). Potent risk factors also include stressful life events, cortisol hypersecretion (*15-18*), and the personality trait neuroticism (*11, 12*).

Serotonergic neurotransmission is a key component of the pathophysiology of depression and mechanism of action of antidepressants. Although the major focus has been on the serotonin transporter, serotonin 2A receptor inhibition and down-regulation, e.g. as seen with SSRIs, mediates antidepressant treatment effects (*19*). Further, genetic variance in the gene encoding the serotonin 2A receptor is associated with outcome of SSRI treatment (*20*). Additional evidence comes from post mortem studies in suicide victims of major depression of which the majority report that serotonin 2A receptor binding is increased in dorsolateral prefrontal cortex (*21, 22*). Although initial findings of in vivo receptor imaging studies were contradictory, two recent studies have confirmed that frontal cortex serotonin 2A receptor binding is

increased, both in recovered, un-medicated patients with a history of major depression (*23*), and in un-medicated patients with severe depression (*24*).

We recently reported that in healthy volunteers frontolimbic 2A receptor binding is positively associated with neuroticism (*25*) and we suggested that this association could be mediated by genetic risk factors of depression. Since both cerebral serotonin 2A receptor level and distribution (*26*) and neuroticism are heritable, and both have been associated with depression, we hypothesized that the correlation between frontolimbic 2A receptor binding and neuroticism may be particularly strong in individuals at high familial risk of developing mood disorders.

We tested the hypothesis that twins at high familial risk of developing mood disorders show a pronounced positive association between frontolimbic serotonin 2A receptor binding and neuroticism, and that this association was either weaker or absent in low-risk twins.

Methods and Materials

Twenty-three healthy high-risk twins with a co-twin diagnosed with mood disorder and 18 low-risk twins whose co-twin did not have the diagnosis were included. They received brain MRI and [^{18}F]-altanserin PET, and were personality assessed and screened for psychiatric symptoms. Three subjects were excluded from analyses: In two cases the measurement of plasma parent compound of [^{18}F]-altanserin failed and in one case the radiochemistry production was too small. Thus, 38 participants were available for analyses: 21 high-risk (12 dizygotic (DZ), 9 monozygotic (MZ)) twins, with familial predisposition through unipolar (17), and bipolar (4) co-twins, mean age 39.3 years (22.1 to 60.9), and 17 low-risk participants (11 DZ, 6 MZ), mean age 38.6 (25.1 to 61.6) years. Female:male ratio was 13:8 (high-risk) and 10:7 (low-risk) reflecting the usual gender distribution in depression. The mean age of onset/diagnosis of the co-twin to high-risk subjects was 32±8.8 years. None of the subjects took psychotrophic drugs. Five women used hormonal contraception (2 high-risk and 3 low-risk subjects) and 1 used hormonal replacement. One used non-sedative antihistamine. None of the subjects used ketanserin, ergots or ergolines.

Healthy high-risk and low-risk twins were identified by linking information from the Danish Twin Registry, the Danish Psychiatric Central Research Register and the Danish Civil Registration System. This linkage identified same-sex twin pairs in which one twin had been treated in a psychiatric hospital setting for an affective episode and the other twin had not (yet) been diagnosed with an affective disorder, the high-risk healthy co-twin. Affected twins were identified as twins who on their first admission, in the period between 1968 and 2005, were discharged from a psychiatric hospital with a diagnosis of depression or recurrent depression (ICD-8-codes 296.09, 296.29, 296.89, 296.99; ICD-10-codes: F32-33.9) or a first diagnosis of manic mixed episode or bipolar affective disorder (ICD-8-Codes 296.19, 296.39; ICD-10-codes: F30-31.6, F34.0 F38.00). From April 1969 to December 1993, diseases were classified according to the International Classification of Diseases,"8th"(ICD-8) (World Health Organization, 1967) and from January 1994 according to the International Classification of Diseases, "10th" (ICD-10) (World Health Organization, 2005). Twins at low risk of developing affective disorders were recruited from a kohort of twin pairs where none of the co-twins had a registered diagnosis or personal history of affective disorder. All subjects gave written informed consent. The Danish

Ministry of Health, The Danish Scientific Ethical Committee ((KF)-12-122/99 and (KF)-01-001/02) and the Danish Data Protection Agency approved the study. The study was conducted in accordance with the latest version of the Declaration of Helsinki.

The participants were part of a larger kohort included in a high-risk study (*27-29*). Of the total cohort of 234 subjects (120 high risk and 114 low risk twins), 100 were invited to participate in a PET-study. Participants and non-participants have been described in details elsewhere (*28*). Criteria for inclusion in the PET-study were no contraindications for MRI, and age below 60 years. Fifty-one % of the invited high-risk and 41% of the low-risk twins agreed to be PET-scanned. Due to a few cancellations caused by technical errors, transport problems, and overweight, 45% of the invited high-risk and 39% of the invited low-risk twins participated. Twelve of the participants from the low-risk group were also included in the study reported in 2007 (*25*).

Clinical data, symptom scores, and personality assessment. Participants were rated in a face-to-face interview by a trained clinician (MV) using semi-structured interviews. Diagnoses were made using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) version 2.1. All persons with a lifetime (current or past) diagnosis of affective disorder, schizoaffective disorder or schizophrenia according to SCAN interview, or significant brain disease were excluded from the study. The Hamilton Depression Scale HAM-D, 17-item (*30*) was used to assess depressive symptoms and self-rating of psychopathology was assessed with the 21-item Beck Depression Inventory (BDI 21) (*31*), and the 14-item Anxiety Subscale (BDI 14) (*32*). No significant differences were found in the prevalence of somatic diagnoses between risk groups and routine blood samples showed no differences between the groups. The assessment is described in detail in Christensen et al (28). Participants were weighed and their heights were measured at the day of the PET-scan. Smoking status was registered.

Participants were asked about stressful life events (SLE) in the year prior to the interview (recent life events), using a Danish version (translated with authorization from the author) of the questionnaires used by Kendler and colleagues (33) as described in detail by Vinberg et al (29).

Participants also completed the Danish version of the 240 item NEO-PI-R (NEO Personality Inventory Revised) self-report personality questionnaire (34) on the same day as the PET scanning. NEO-PI-R evaluates the broad personality dimensions of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness. Each dimension or factor score is derived by adding the scores from assessment of six constituent personality traits (facets) and each trait score is derived by adding the scores on 8 items in 0 – 4 Likert format (*35*). The overall neuroticism factor score was the measure used in the study design. Post hoc, the contribution from the constituent traits of neuroticism (anxiety, depression, self-conscientiousness, vulnerability, impulsiveness, and angry hostility) was evaluated.

Imaging. The serotonin 2A receptor binding was imaged with [^{18}F]altanserin PET according to Pinborg et *al*. (*36*). After bolus-infusion of tracer for 2 hours to attain tracer steady state conditions, 40 minutes emission scans were acquired with an eighteen-ring GE-Advance scanner (GE, Milwaukee, Wisconsin, USA) operating in 3D-acquisition mode. The total axial field of view was 15.2 cm with an approximate in-plane resolution of 6 mm. During scanning, the fraction of un-metabolized tracer in venous plasma was determined at 3 time points using high performance liquid chromatography analysis (HPLC). Reconstruction, including attenuation correction, and scatter correction are described in detail elsewhere (*36*). Subjects received a maximum dose of 3.7 MBq/kg bodyweight [^{18}F]altanserin. Structural magnetic resonance (MR) brain imaging was conducted in all subjects; MPRAGE sequences were acquired on a 3 T scanner (Trio, Siemens, Erlangen, Germany).

MR/PET co-registration. PET and MR images were co-registered through manual translation and rotation of the PET image with subsequent visual inspection in three planes, using a Matlab (Mathworks Inc., Natick, MA, USA) based program, as described in Adams et al. (37). Data analyses of all PET- and MR-scans were carried out by the same person.

Volumes of interest (VOIs) and partial volume correction. Based on the method by Svarer et al. (38) a frontolimbic volume was automatically delineated on each individual's transaxial MRI slices in a strictly user-independent fashion. This method utilizes a VOI probability map based on a template set of ten MRIs, where VOIs have been defined manually. After alignment, the VOIs were transferred onto the PET

images. The frontolimbic volume included the orbitofrontal, medial inferior frontal, superior frontal, anterior cingulate, posterior cingulate, hippocampus, and entorhinal cortices.

To enable partial volume correction of the PET data, MR images were segmented into gray matter, white matter, and cerebrospinal fluid tissue classes using Statistical Parametric Mapping (SPM2; Wellcome Department of Cognitive Neurology, London, UK). Partial volume correction was performed according to Muller-Gartner, as defined in Quarantelli et al (*39*).

Quantification of the serotonin 2A receptor binding. The outcome parameter was the binding potential of specific tracer binding (BP_P). Cerebellum was used as a reference region since it represents non–specific binding only. BP_P is defined as the difference between total (C_{ROI}) and non-specific binding ($C_{Reference}$), in steady state, divided by the steady state activity of non-metabolized tracer in plasma (C_{Plasma}).

$$BP_P = (C_{VOI} - C_{Reference}) / C_{Plasma} = f_p * (B_{max}/K_d) \text{ (mL/mL)}$$

where C_{VOI} and $C_{Reference}$ are mean counts in the VOI and in the reference region, respectively, C_{Plasma} is the radioactivity originating from parent compound in plasma, f_P is the free fraction of radiotracer, B_{max} is the density of receptor sites available for tracer binding, and K_d is the affinity constant of the radiotracer to the receptor (*36*).

Statistics. Group comparisons of clinical data were performed using two-sided, unpaired t-tests. Differences in proportions were tested with the Fischer's Exact test.

Main effects of neuroticism, risk-status, gender, smoking, symptom scores, and SLE on frontolimbic serotonin 2A receptor binding were tested by multiple linear regression analysis (two-sided test) in a model adjusting for age and body mass index (BMI), known to influence serotonin 2A receptor binding (*37*).

To test whether the association between frontolimbic serotonin 2A receptor binding and neuroticism was most pronounced in the group at high familial risk, we evaluated the effect of "neuroticism by risk" interaction in a multiple linear regression analysis with frontolimbic serotonin 2A receptor binding as the outcome parameter and age, BMI, neuroticism, risk-status as covariates. The effect of "zygosity by risk" was also tested assuming that a genetic effect would be most prominent in MZ high-risk subjects followed by DZ high-risk, and DZ/MZ low-risk subjects. Post hoc, the

effect of “gender by risk” interaction was also tested since some genetic risk factors are likely to be sex-specific in their effect (*7*).

Linearity of quantitative variables was confirmed by including second order terms in the models. Variance homogeneity and normality were assured by graphical evaluation. Accordingly, model assumptions were met.

The hypothesized interaction between neuroticism and risk-status was tested by a one-sided test, since the direction was anticipated from our previous study (Frokjaer et al, 2008). P-values, parameter estimates with standard errors (SE) and 95% confidence limits, and degrees of freedom (DF) are reported when appropriate. The analyses were performed in R 2.5.1 (http://www.R-project.org).

Results

Group differences and main effects of clinical data, symptom scores, and neuroticism

Demographical data and symptom scores for high- and low-risk twins are given in table 1. The only statistically significant difference between the groups was that the high-risk group smoked more (p=0.03).

Neuroticism tended to correlate with frontolimbic serotonin 2A receptor binding in a simple model adjusting for age and BMI, but disregarding risk-status (p=0.06). We saw no main effects of risk-status, gender, smoking, symptom scores, or SLE on frontolimbic serotonin 2A receptor binding when adjusting for age and BMI (table 2). Further, we observed no effects of bi- versus uni-polar co-twin history, years passed from age of onset/diagnosis of co-twin, or age of onset/diagnosis of the co-twin (table 2).

Effect of risk group on the association between frontolimbic serotonin 2A receptor binding and neuroticism

To explore whether the risk-status influenced the association between frontolimbic serotonin 2A receptor binding and neuroticism, we tested the interaction between neuroticism and risk-status in a model including BMI, age, neuroticism, risk-status, and neuroticism times risk.

As illustrated in figure 1, we saw an interaction between neuroticism and risk in the expected direction (difference in slope between high-risk and low-risk: 0.022 BP_P, (SE: 0.11, lower bound of one-sided 95% confidence interval: 0.0029 BP_P), p=0.030, DF:32). The high-risk group showed a more pronounced positive association between neuroticism and frontolimbic serotonin 2A receptor binding than the low-risk group, (high-risk: 0.026 BP_P per neuroticism unit (SE: 0.0095, lower bound of one-sided 95% confidence interval: 0.010 BP_P, p=0.0049, DF:32), low-risk: 0.0047 BP_P per neuroticism unit (SE: 0.0065, lower bound of one-sided 95% confidence interval: -0.0063 BP_P, p=0.25, DF: 32)).

When adding zygosity to the model of interaction between risk and neuroticism, the 4 groups of increasing risk-load (low-risk MZ/DZ < high-risk DZ < high-risk MZ) showed the expected hierarchy in terms of increasing association

between frontolimbic serotonin 2A and neuroticism, except for the two low-risk groups that were almost equal (slope estimates: 0.0045/0.0037, 0.018, and 0.028, respectively, DF:28). The hierarchy represented a trend only.

The association between neuroticism and serotonin 2A receptor binding in the high-risk group was primarily driven by the constituent traits self-conscientiousness (p=0.005), anxiety (p=0.020), depression (p=0.025), and less convincingly by vulnerability (p=0.12), whereas little or no association was seen with impulsivity (p=0.24) or angry hostility (p=0.27), DF:17.

Effects of gender-risk interaction in the association between frontolimbic 2A and neuroticism

In high-risk females, the association between frontolimbic serotonin 2A receptor binding and neuroticism was more pronounced than in the remaining group (difference in slope: 0.027 BP_P per neuroticism unit (SE: 0.012, two-sided 95% confidence limits: 0.020 – 0.053), p=0.036, DF:32). Within the group of 13 high-risk females the effect of neuroticism on frontolimbic serotonin 2A receptor binding adjusted for age and BMI was substantial (0.033 BP_P per neuroticism unit, (SE: 0.011, two-sided 95% confidence limits: 0.010 – 0.056), p=0.0050).

Discussion

We have demonstrated that the coupling between frontolimbic serotonin 2A receptor binding and neuroticism is more pronounced in individuals at high familial risk for developing depression than in individuals at low risk. This finding confirms the hypothesis, that familial risk for developing mood disorders mediates an association between high frontolimbic serotonin 2A receptor binding and high neuroticism. Because of the limited sample size of MZ vs. DZ twins, a traditional analysis of genetic predisposition versus shared early environmental factors cannot be undertaken, but this was not the aim of the study. We did, however, see that the groups of increasing risk-load (low-risk MZ/DZ < high-risk DZ < high-risk MZ) show the expected hierarchy in terms of increasing association between frontolimbic serotonin 2A and neuroticism.

Our findings support that familial risk of depression is associated with frontolimbic serotonin 2A receptor binding in a neuroticism dependent manner. A recent study by Bhagwagar et al (*23*), in un-medicated, euthymic patients recovered from depression, support that a higher frontolimbic serotonin 2A receptor level may be a trait factor of the susceptibility to develop depression. Also, Meyer et al found that in patients with a major depressive episode (*24*) cortical, predominantly frontal, serotonin 2 receptor binding was correlated to dysfunctional attitudes (pessimistic, negativistic thinking), whereas only the subgroup of patients with the most severe depression had higher frontal serotonin 2 receptor binding than the healthy controls. In none of these studies neuroticism was, however, taken into account. High frontal serotonin 2A receptor in depression is also well documented in postmortem studies (*21*). However, initial in vivo imaging studies were contradictory. Three studies sampling subjects recently influenced by antidepressant treatment (*40-42*) and a study sampling subjects exposed to benzodiazepines (*43*) reported decreased serotonin 2A receptor binding in depression. Two studies, not biased by treatment effects, reported no changes (*44, 45*). Accordingly, treatment effects, and in addition, limited sample sizes and the use of non-selective serotonin 2 receptor tracers may have confounded these initial findings.

Our finding of increased serotonin 2A receptor binding is linked to familial and personality risk factors of depression is well in line with the observation that central serotonin 2A agonism stimulates cortisol excretion (*46*), and it may also reflect

the observation that enhanced cortical serotonin 2A signaling in rodents is accompanied by a tendency to perceive or judge an environment as risky (*47*). Indeed, the combination of high frontolimbic serotonin 2A receptor binding and high neuroticism might be particularly disadvantageous in the context of stress since a strong neuroticism personality component will shape the perception and enhance the negative impact of stress (*48*). The correlation between serotonin 2A receptor binding, neuroticism, and genetic predisposition may reflect serotonergic modulations in early brain development since serotonin plays a critical role in the maturation of brain systems that modulate emotional function in the adult (*49*).

The increase in frontolimbic serotonin 2A receptor levels may be primary or an up-regulation of serotonin 2A receptor levels could occur in response to genetically or environmentally induced low serotonin levels (*50-52*) or high cortisol excretion (*53*).

As earlier observed, some constituent traits of neuroticism contribute more to the association between serotonin 2A receptor binding and neuroticism (*25*). Whereas we did not see such a strong relationship with vulnerability, as in our previous study of healthy volunteers (*25*), a subset of the neuroticism trait consisting of the facets self-consciousness, depression, anxiety, and vulnerability contributed to the association in subjects at high familial risk. While these traits inherently reflect sensitivity to stress and trauma, the remaining two facets of neuroticism, angry hostility and impulsiveness, clearly reflect a disposition to more active reaction when faced with frustration. Interestingly, in the present study, in an independent sample, we confirmed our previous finding that angry hostility and impulsiveness facets of neuroticism do not contribute to the correlation between high frontolimbic serotonin 2A receptor binding and neuroticism. Therefore, we suggest that angry hostility and impulsiveness do not significantly contribute to the personality related risk for developing depressive disorder and that the subset of the neuroticism trait is a more sensitive risk marker.

Neuroticism mean score and variance did not differ significantly between twins at high versus low risk and hence, the more pronounced association in the high-risk group is not driven by a larger variance in neuroticism score. In the cohort of 211 healthy twins only a modestly increased neuroticism score was found in high-risk twins but when gender, minor psychopathology and effects of SLE were taken into account, there was no difference in neuroticism score (*29*).

There were significantly more current smokers in the high-risk group than in the low-risk group. This is well in line with the observation that people with current or past depression are more likely to have been smokers at some point in their lives (*54*). The association between genetic predisposition to depression and addiction to smoking might even reflect common genetic factors between smoking addiction and risk for developing depression (*54*). Smoking may potentially lead to alterations in frontal serotonin 2A receptor binding through adaptation to increased central serotonin release. Such alterations would be expected to be towards a lower serotonin 2A receptor level in smokers. However, in a large sample of healthy volunteers we did not observe any effect of smoking on brain serotonin 2A receptor binding (*55*).

Methodological considerations

Our results should be interpreted in the context of some potentially significant methodological considerations.

Firstly, the high-risk group consisted of subjects with variable risk-load and several aspects potentially contributed to reduce the risk.

a. Average age of diagnosis of the co-twin was 32.2 ± 8.8 years, with 3 affected co-twins being older than 40 years (43, 47, 54). Later age of onset would be anticipated to be associated with lower risk.

b. In the high-risk twins a large variation in the number of years between inclusion in our study and date of onset of the co-twins mood disorder was present. The mean symptom free interval was 7.0±7.6 years, but for 3 subjects more than 10 years passed from the age of onset of the co-twin (10, 20, and 34 years). These subjects did, despite their predisposition, for a long time not develop mood disorders and may therefore be protected. We did not, however, observe a correlation between depression free years from age of onset and frontolimbic serotonin 2A binding.

c. Pooling of unipolar and bipolar predisposition. The fraction of predisposition through bipolar co-twins was moderate in our sample: 19% (17 unipolar and 4 bipolar). Since the frequency of unipolar depression in relatives of unipolar and bipolar patients is the same (*56*) we do not expect that this has influenced the risk. Moreover, it is not clear whether unipolar and bipolar disorder are categorically distinct or whether they lie on a continuous spectrum (*57*).

d. Pooling across gender. Heritability of depression seems slightly more pronounced in women than in men, and depression is twice as frequent in women (*7*). In our study

only same-sex twin pairs were included. Even though gender did not significantly affect the serotonin 2A receptor binding, we observed a "gender by risk" interaction suggesting a gender-related effect of genetic risk factors on the coupling between frontolimbic serotonin 2A receptor binding and neuroticism. This finding will need to be replicated.

e. We expect that subjects volunteering for an extensive investigation program, including a PET-study, would tend to be more robust.

Since several of the factors mentioned above tend to reduce the risk in the high-risk group it is possible that an even more pronounced association between frontolimbic serotonin 2A receptor binding and neuroticism could have been found in a high-risk group more selected towards high risk.

Secondly, it could be argued that twin sample may not be representative of singletons. However, several studies support that depression in twins is typical to that of the general population. Both monozygotic and dizygotic twins are typical of the general population in their risk for treated affective disorders (*58, 59*), and are also typical with respect to the level and variability of self-reported psychiatric symptoms (*60*).

In conclusion, the associations between familial risk, frontolimbic serotonin 2A receptor binding, and neuroticism are complex. Our data suggest that familial risk of developing mood disorders mediates an association between high frontolimbic serotonin 2A receptor binding and high neuroticism. We speculate that the combination of high frontolimbic serotonin 2A receptor binding and high neuroticism might be particularly disadvantageous in the context of mood disorders, by enhancing the negative impact of stress.

ACKNOWLEDGEMENTS

We are grateful to the participants. We wish to thank Karin Stahr, Dorthe Givard and the staff at the PET centre, Rigshospitalet for their superb technical assistance. Thanks to the Department of Psychiatric Demography, University of Aarhus, Psychiatric Hospital, Riskov, Denmark, and to the Danish Twin Registry for cooperation in the study. The John and Birthe Meyer Foundation is thanked for the donation of the Cyclotron and PET scanner. This work was generously supported by Danish Medical Research Council, The Health Science Faculty, University of Copenhagen, the 1991 Pharmacy Foundation, The Lundbeck Foundation, the EU Sixth framework programme (EC-FP6-project DiMI (LSHB-CT-2005-512146), Sawmill owner Jeppe Juhl and Wife Ovita Juhls Foundation, "Laegernes forsikringsforening af 1891", and "Fonden af 1870".

COMPETING INTEREST STATEMENT

The authors declare no competing financial interest.

Table 1. Comparison of clinical data

	High-risk N=21	*Low-risk N=17*	*p-value*
Age (years)	39.3±11.9	38.6±11.7	p=0.86
Smoking	48%	12%	p=0.034
Alcohol (units/week)	7.1±8.0	3.7±2.9	p=0.11
Body mass index	23.9±3.1	25.5±3.0	p=0.12
Education years	12.7±2.8	13.7±2.5	p=0.28
Hamilton	2.7±1.3	2.4±1.5	p=0.55
BDI depression	2.2±2.5	1.2±1.24	p=0.15
BDI anxiety	0.9±1.4	1.3±1.5	p=0.41
Neuroticism	71±14	67±23	p=0.49
SLE, 3 or more	40%	25%	p=0.48
Twin age of onset	32.2±8.8	NA	NA
Years without diagnosis	7.0±7.6	NA	NA

BDI_depression: Becks Depression Inventory, depression symptoms component (21 items). BDI_anxiety: anxiety component (14 items). Hamilton: Hamilton Depression rating Scale score of depressive symptoms (17 items). SLE: the fraction of participants experiencing 3 or more stress-full life events within the last 12 months. Neuroticism: score from 240-item NEO-PI-R, Danish version. Body mass index= weight/ height^2 (kg/m^2). Smoking: proportion of smokers. Years without diagnosis: number of years passed from age of onset of mood disorder of co-twin and the PET-scan. Statistics: Group differences in numerical variables were tested by unpaired t-tests, differences in proportions by Fischer's exact test. P-values are reported with no corrections for multiple comparisons. NA: not applicable.

Table 2. Main effects of clinical variables in a model adjusting for age and BMI

Variable	*Estimate*	*SE*	*95% Confidence limits*	*p-value*	*DF*
Gender	0.25	0.20	(-0.17 – 0.67)	p=0.23	34
Smoking	0.098	0.21	(-0.34 – 0.53)	p=0.65	34
BDI_depression	0.0093	0.053	(-0.098 – 0.12)	p=0.86	32
BDI_anxiety	-0.027	0.073	(-0.18 – 0.12)	p=0.71	32
Hamilton	-0.10	0.073	(-0.25 – 0.047)	p=0.17	33
SLE-3or more	-0.19	0.23	(-0.66 – 0.27)	p=0.41	32
Neuroticism	0.011	0.0057	(-0.00055 – 0.023)	p=0.06	34
Risk-status	-0.055	0.21	(-0.48 – 0.37)	p=0.79	34
Bi or unipolar	0.0062	0.37	(-0.78 – 0.79)	p=0.99	17
Age of onset	-0.006	0.027	(-0.063 – 0.051)	p=0.83	17
“Years passed”	0.0060	0.027	(-0.051- 0.063)	p=0.83	17

Gender: women>men. Smoking: smoker>non-smoker. SLE_3or more: 3 or more>0,1,2 SLE. Risk-status: high-risk>low-risk. The effects of diagnoses and age of onset concerns the high-risk group only. Bi or unipolar diagnoses of co-twin: diagnosis at first admission. Age of onset: age of first diagnosis. Years passed: number of years passed since age of onset of mood disorder of co-twin to PET-scan. DF: degrees of freedom.

Figure 1

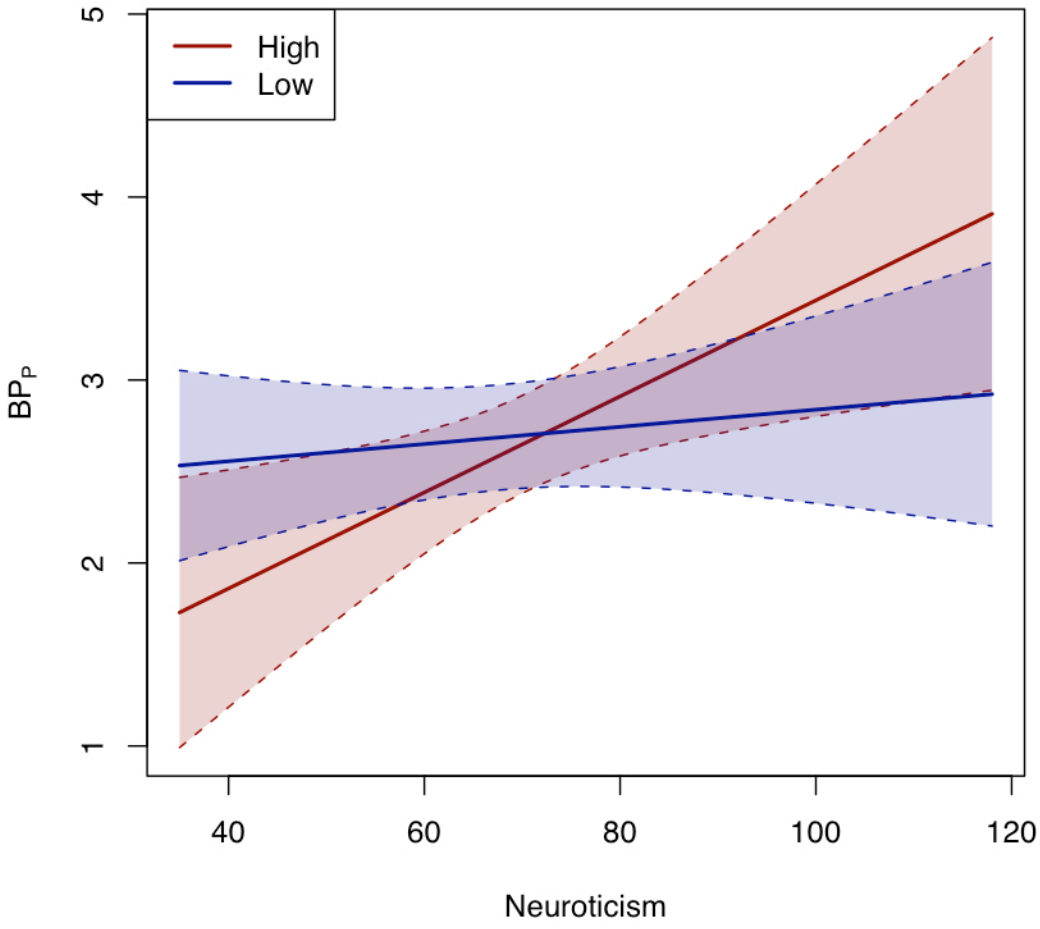


Figure 1. Effect of risk status on the association between frontolimbic 2A receptor BP_P and neuroticism, adjusted for BMI and age. In the high-end of the neuroticism scale, the high-risk group showed an elevated BP_P, whereas, in the low-end they showed a decreased BP_P as compared to the low-risk group. This reflects the pronounced coupling between neuroticism and frontolimbic 2A receptor binding in twins at high genetic risk of developing mood disorders, p=0.03, see text. Point-wise 95% symmetric confidence bands of the regression lines are displayed. The regression lines represent the associations given a mean BMI and mean age.

Figure 2

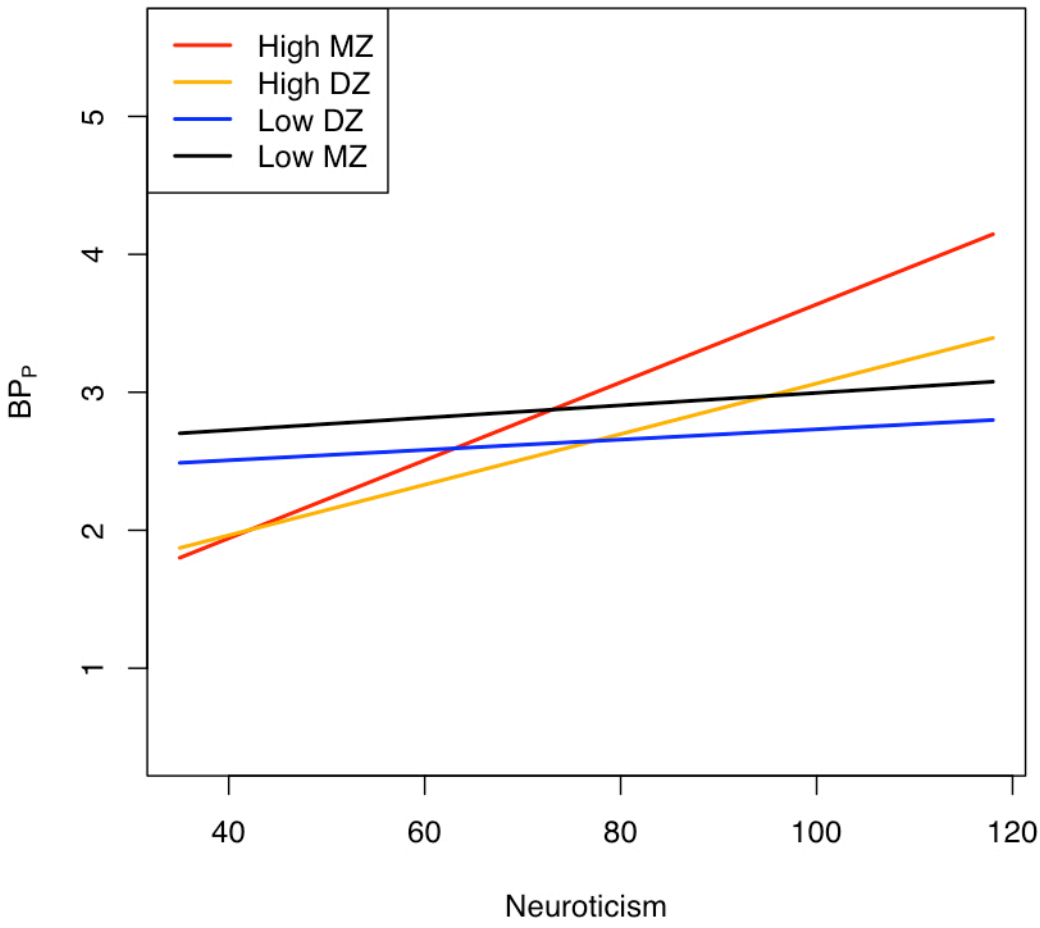


Figure 2. Effect of zygosity and risk on the association between frontolimbic 2A receptor binding and neuroticism, adjusting for BMI and age. Genetic risk-load: (low-risk MZ < low-risk DZ < high-risk DZ < high-risk MZ). The high-risk MZ twins show the most pronounced association, followed by the high-risk-DZ group.

References

1. Lyons MJ, Eisen SA, Goldberg J, True W, Lin N, Meyer JM, et al. (1998): A registry-based twin study of depression in men. *Arch Gen Psychiatry*. 55:468-472.
2. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ (1993): The lifetime history of major depression in women. Reliability of diagnosis and heritability. *Arch Gen Psychiatry*. 50:863-870.
3. Kendler KS, Gardner CO, Prescott CA (1999): Clinical characteristics of major depression that predict risk of depression in relatives. *Arch Gen Psychiatry*. 56:322-327.
4. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. (2003): Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. 301:386-389.
5. Sullivan PF, Neale MC, Kendler KS (2000): Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry*. 157:1552-1562.
6. McGuffin P, Katz R, Rutherford J (1991): Nature, nurture and depression: a twin study. *Psychol Med*. 21:329-335.
7. Kendler KS, Gatz M, Gardner CO, Pedersen NL (2006): A Swedish national twin study of lifetime major depression. *Am J Psychiatry*. 163:109-114.
8. Brummett BH, Boyle SH, Siegler IC, Kuhn CM, Ashley-Koch A, Jonassaint CR, et al. (2007): Effects of Environmental Stress and Gender on Associations among Symptoms of Depression and the Serotonin Transporter Gene Linked Polymorphic Region (5-HTTLPR). *Behav Genet*.
9. Barr CS, Newman TK, Schwandt M, Shannon C, Dvoskin RL, Lindell SG, et al. (2004): Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. *Proc Natl Acad Sci U S A*. 101:12358-12363.
10. Levinson DF (2006): The genetics of depression: a review. *Biol Psychiatry*. 60:84-92.
11. Fanous AH, Neale MC, Aggen SH, Kendler KS (2007): A longitudinal study of personality and major depression in a population-based sample of male twins. *Psychol Med*. 37:1163-1172.
12. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ (1993): A longitudinal twin study of personality and major depression in women. *Arch Gen Psychiatry*. 50:853-862.
13. Kendler KS, Gatz M, Gardner CO, Pedersen NL (2006): Personality and major depression: a Swedish longitudinal, population-based twin study. *Arch Gen Psychiatry*. 63:1113-1120.
14. Fanous A, Gardner CO, Prescott CA, Cancro R, Kendler KS (2002): Neuroticism, major depression and gender: a population-based twin study. *Psychol Med*. 32:719-728.
15. Mannie ZN, Harmer CJ, Cowen PJ (2007): Increased waking salivary cortisol levels in young people at familial risk of depression. *Am J Psychiatry*. 164:617-621.
16. Portella MJ, Harmer CJ, Flint J, Cowen P, Goodwin GM (2005): Enhanced early morning salivary cortisol in neuroticism. *Am J Psychiatry*. 162:807-809.
17. Modell S, Lauer CJ, Schreiber W, Huber J, Krieg JC, Holsboer F (1998): Hormonal response pattern in the combined DEX-CRH test is stable over time in subjects at high familial risk for affective disorders. *Neuropsychopharmacology*. 18:253-262.
18. Bhagwagar Z, Hafizi S, Cowen PJ (2003): Increase in concentration of waking salivary cortisol in recovered patients with depression. *Am J Psychiatry*. 160:1890-1891.
19. Gray JA, Roth BL (2001): Paradoxical trafficking and regulation of 5-HT(2A) receptors by agonists and antagonists. *Brain Res Bull*. 56:441-451.
20. McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, et al. (2006): Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *Am J Hum Genet*. 78:804-814.
21. Stockmeier CA (2003): Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. *J Psychiatr Res*. 37:357-373.
22. Arango V, Underwood MD, Mann JJ (1997): Postmortem findings in suicide victims. Implications for in vivo imaging studies. *Ann N Y Acad Sci*. 836:269-287.
23. Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P (2006): Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL 100,907. *Am J Psychiatry*. 163:1580-1587.
24. Meyer JH, McMain S, Kennedy SH, Korman L, Brown GM, DaSilva JN, et al. (2003): Dysfunctional attitudes and 5-HT2 receptors during depression and self-harm. *Am J Psychiatry*. 160:90-99.
25. Frokjaer VG, Mortensen EL, Nielsen FA, Haugbol S, Pinborg LH, Adams KH, et al. (2007): Frontolimbic Serotonin 2A Receptor Binding in Healthy Subjects Is Associated with Personality Risk Factors for Affective Disorder. *Biol Psychiatry*.

26. Pinborg L, Arfan H, Haugbol S, Kyvik K, Hjelmborg J, Svarer C, et al. (2007): The density of 5-HT_{2A} receptors in the human brain is strongly genetically determined. *Neuroimage*. doi:10.1016/j.neuroimage.2007.09.019.
27. Christensen MV, Kyvik KO, Kessing LV (2006): Cognitive function in unaffected twins discordant for affective disorder. *Psychol Med*. 36:1119-1129.
28. Christensen MV, Kyvik KO, Kessing LV (2007): Subclinical psychopathology and socio-economic status in unaffected twins discordant for affective disorder. *J Psychiatr Res*. 41:229-238.
29. Vinberg M, Mortensen EL, Kyvik KO, Kessing LV (2007): Personality traits in unaffected twins discordant for affective disorder. *Acta Psychiatr Scand*. 115:442-450.
30. Hamilton M (1967): Development of a rating scale for primary depressive illness. *Br J Soc Clin Psychol*. 6:278-296.
31. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961): An inventory for measuring depression. *Arch Gen Psychiatry*. 4:561-571.
32. Beck AT, Epstein N, Brown G, Steer RA (1988): An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol*. 56:893-897.
33. Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B (2005): The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch Gen Psychiatry*. 62:529-535.
34. Skovdahl-Hansen H, Mortensen, EL., Scioetz, HK. (2004): Dokumentation for den danske udgave af NEO PI-R og NEO PI-R Kort Version. Copenhagen, Denmark: Dansk psykologisk forlag.
35. Costa PT, McCrae RR (1992): Professional manual for Revised NEO Personality Inventory. Odessa FL: Psychological Assessment Resources.
36. Pinborg LH, Adams KH, Svarer C, Holm S, Hasselbalch SG, Haugbol S, et al. (2003): Quantification of 5-HT2A receptors in the human brain using [18F]altanserin-PET and the bolus/infusion approach. *J Cereb Blood Flow Metab*. 23:985-996.
37. Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S, et al. (2004): A database of [(18)F]-altanserin binding to 5-HT(2A) receptors in normal volunteers: normative data and relationship to physiological and demographic variables. *Neuroimage*. 21:1105-1113.
38. Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, et al. (2005): MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage*. 24:969-979.
39. Quarantelli M, Berkouk K, Prinster A, Landeau B, Svarer C, Balkay L, et al. (2004): Integrated software for the analysis of brain PET/SPECT studies with partial-volume-effect correction. *J Nucl Med*. 45:192-201.
40. Attar-Levy D, Martinot JL, Blin J, Dao-Castellana MH, Crouzel C, Mazoyer B, et al. (1999): The cortical serotonin2 receptors studied with positron-emission tomography and [18F]-setoperone during depressive illness and antidepressant treatment with clomipramine. *Biol Psychiatry*. 45:180-186.
41. Biver F, Wikler D, Lotstra F, Damhaut P, Goldman S, Mendlewicz J (1997): Serotonin 5-HT2 receptor imaging in major depression: focal changes in orbito-insular cortex. *Br J Psychiatry*. 171:444-448.
42. Yatham LN, Liddle PF, Shiah IS, Scarrow G, Lam RW, Adam MJ, et al. (2000): Brain serotonin2 receptors in major depression: a positron emission tomography study. *Arch Gen Psychiatry*. 57:850-858.
43. Messa C, Colombo C, Moresco RM, Gobbo C, Galli L, Lucignani G, et al. (2003): 5-HT(2A) receptor binding is reduced in drug-naive and unchanged in SSRI-responder depressed patients compared to healthy controls: a PET study. *Psychopharmacology (Berl)*. 167:72-78.
44. Meyer JH, Kapur S, Houle S, DaSilva J, Owczarek B, Brown GM, et al. (1999): Prefrontal cortex 5-HT2 receptors in depression: an [18F]setoperone PET imaging study. *Am J Psychiatry*. 156:1029-1034.
45. Meltzer CC, Price JC, Mathis CA, Greer PJ, Cantwell MN, Houck PR, et al. (1999): PET imaging of serotonin type 2A receptors in late-life neuropsychiatric disorders. *Am J Psychiatry*. 156:1871-1878.
46. Van de Kar LD, Javed A, Zhang Y, Serres F, Raap DK, Gray TS (2001): 5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate hypothalamic CRF and oxytocin-expressing cells. *J Neurosci*. 21:3572-3579.
47. Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP, et al. (2006): Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. *Science*. 313:536-540.

48. Jacobs N, Kenis G, Peeters F, Derom C, Vlietinck R, van Os J (2006): Stress-related negative affectivity and genetically altered serotonin transporter function: evidence of synergism in shaping risk of depression. *Arch Gen Psychiatry*. 63:989-996.
49. Gaspar P, Cases O, Maroteaux L (2003): The developmental role of serotonin: news from mouse molecular genetics. *Nat Rev Neurosci*. 4:1002-1012.
50. Stockmeier CA, Kellar KJ (1986): In vivo regulation of the serotonin-2 receptor in rat brain. *Life Sci*. 38:117-127.
51. Roth BL, Berry SA, Kroeze WK, Willins DL, Kristiansen K (1998): Serotonin 5-HT2A receptors: molecular biology and mechanisms of regulation. *Crit Rev Neurobiol*. 12:319-338.
52. Cahir M, Ardis T, Reynolds GP, Cooper SJ (2007): Acute and chronic tryptophan depletion differentially regulate central 5-HT1A and 5-HT 2A receptor binding in the rat. *Psychopharmacology (Berl)*. 190:497-506.
53. Dwivedi Y, Mondal AC, Payappagoudar GV, Rizavi HS (2005): Differential regulation of serotonin (5HT)2A receptor mRNA and protein levels after single and repeated stress in rat brain: role in learned helplessness behavior. *Neuropharmacology*. 48:204-214.
54. Kendler KS, Neale MC, MacLean CJ, Heath AC, Eaves LJ, Kessler RC (1993): Smoking and major depression. A causal analysis. *Arch Gen Psychiatry*. 50:36-43.
55. Erritzoe D, Frokjaer V, Arfan H, Haugbol S, Pinborg L, Svarer C, et al. (2006): Overweight associated with increased serotonin 2A receptor binding in humans. *J Nucl Med*. 47 suppl.1:7P.
56. Kutcher S, Marton P (1991): Affective disorders in first-degree relatives of adolescent onset bipolars, unipolars, and normal controls. *J Am Acad Child Adolesc Psychiatry*. 30:75-78.
57. Akiskal HS, Benazzi F (2006): The DSM-IV and ICD-10 categories of recurrent [major] depressive and bipolar II disorders: evidence that they lie on a dimensional spectrum. *J Affect Disord*. 92:45-54.
58. Kendler KS, Pedersen NL, Farahmand BY, Persson PG (1996): The treated incidence of psychotic and affective illness in twins compared with population expectation: a study in the Swedish Twin and Psychiatric Registries. *Psychol Med*. 26:1135-1144.
59. Klaning U, Laursen TM, Licht RW, Kyvik KO, Skytthe A, Mortensen PB (2004): Is the risk of bipolar disorder in twins equal to the risk in singletons? A nationwide register-based study. *J Affect Disord*. 81:141-145.
60. Kendler KS, Martin NG, Heath AC, Eaves LJ (1995): Self-report psychiatric symptoms in twins and their nontwin relatives: are twins different? *Am J Med Genet*. 60:588-591.

Paper 3

High familial risk for mood disorder is associated with low dorsolateral prefrontal cortex serotonin transporter binding

Vibe G. Frokjaer[1], Maj Vinberg[2], David Erritzoe[1], Claus Svarer[1], Wiliam Baare[4], Karine Madsen[1], Jacob Madsen[3], Lars V. Kessing[2], Gitte M. Knudsen[1]

[1]Neurobiology Research Unit and Center for Integrated Molecular Brain Imaging, [2]Department of Psychiatry, and [3]PET and Cyclotron Unit Copenhagen University Hospital Rigshospitalet, [4]Danish Research Centre for Magnetic Resonance, Hvidovre University Hospital, University of Copenhagen, Denmark

Corresponding author:
Vibe G. Frokjaer, M.D.
Neurobiology Research Unit 9201
Copenhagen University Hospital, Rigshospitalet
Blegdamsvej 9, DK-2100 Copenhagen, Denmark
Telephone: +45 35456712, Fax: +45 356713.
Email: vibe@nru.dk

Abstract

Context: Mood disorders are elicited through a combination of genetic and environmental stress factors, and treatment with selective serotonin reuptake inhibitors ameliorates depressive symptoms. Changes in the serotonin transporter (SERT) binding may therefore occur in depressive patients and in subjects at risk for developing depression.

Objectives: To explore if familial predisposition to depression is associated with changes in brain SERT binding, as a trait marker of mood disorder.

Design: High-risk – low-risk study.

Setting: Healthy twins at high risk and at low risk were identified by linking information from the Danish Twin Register and the Danish Psychiatric Central Register.

Participants: Nine healthy twins (5 DZ and 4 MZ), mean age 32.2±4.2 years, at high risk of mood disorder and 11 twins at low risk (8 DZ, 3 MZ), mean age 32.4±5.0 years.

Main outcome measure: Regional in vivo brain serotonin transporter binding was measured with [^{11}C]DASB PET.

Results: The depression symptom scores of the high and the low risk twins were not significantly different. The twins at high risk for mood disorders had a 35% reduction in SERT binding in dorsolateral prefrontal cortex ($p=0.0018$) and a 15% reduction in anterior cingulate ($p=0.018$).

Conclusion: Our data suggest that a low SERT binding in dorsolateral prefrontal cortex and anterior cingulate represents a trait marker for mood disorders.

Introduction

The most potent risk factor for mood disorder is a relevant family history. In particular, recurrence and early age of onset characterize familial cases[1-3]. Twin studies suggest that the genetic heritability of depression is approximately 40%[2, 4-6]. The disorder is predominantly polygenetically inherited[7]; in a recent genome-wide linkage study 3 chromosome regions containing genes with possible contributions to the risk of developing depression have been identified[8]. Further, in a seminal paper by Caspi et al[9] it was documented that the low expressing s-variant of the 5-HTLPR polymorphism in the promoter region of the SERT gene in combination with stressful life events greatly enhanced the risk of developing major depression. Whether the increased vulnerability to depression in carriers of the "s" allele is mediated through a direct effect on in vivo SERT availability at any time during life is not yet clear[10, 11]. Early exposure to stress, such as in fetal life, possibly induces a long-lasting increased responsiveness to stress and is associated with increased risk of later development of depression[12-14]. Serotonergic alterations early in life thus play a role since serotonin is critical in the maturation of brain systems that modulate emotional function in the adult[15].

Since selective pharmacological inhibition of SERT is associated with amelioration of depressive symptoms[16] dysregulation of serotonergic transmission plays a key role in the pathophysiology of depression. Further, people with a family history of affective disorder are more prone to develop depressive symptoms when depleted of brain tryptophan, the precursor of serotonin[17, 18]. This suggests a genetic vulnerability to serotonergic dysfunction.

As reviewed by Stockmeier, most postmortem studies in depressed and/or suicide subjects show either a decreased or no change in prefrontal SERT binding, and normal brainstem SERT binding[19]. In vivo imaging studies of the SERT in mood disorders show mixed results in *subcortical* regions observing either decreased[20-23], no changes[24, 25], or increased SERT binding[25-29]. The mixed results may reflect differences in radiotracer properties, of the applied quantification methods, age effects, clinical heterogeneity of patients, limited samples size, or antidepressant treatment effects. Only with the recent introduction of PET tracers such as [^{11}C]MADAM and [^{11}C]DASB that both are characterized by a high selectivity for SERT and a favourable ratio of specific binding relative to non-specific binding[30], in

vivo imaging of *cortical* SERT levels has become possible. [^{11}C]DASB studies of patients with ongoing depression have shown unchanged or elevated SERT-binding levels in cortical regions[24, 25, 28]. At present, no studies have investigated brain SERT binding in healthy subjects at high versus low risk for mood disorders. Given that mood disorders are highly heritable, changes in SERT binding may constitute a trait marker for the development of a mood disorder.

Studies of twins are particularly valuable in affective disorders, since twins share both early environmental factors and also a large fraction of their genes. A study of healthy twins at high risk versus low risk would yield maximal contrast and would also avoid confounding effects of prior or current psychiatric disorder, including effects of antidepressant treatment. We tested the hypothesis that familial risk of developing mood disorders is associated with changes in SERT binding in brain regions relevant for this condition.

Methods and Materials

Nine healthy high-risk twins, 5 dizygotic (DZ) and 4 monozygotic (MZ), mean age 32.2±4.2 years (range 27-40) with a co-twin history of mood disorder (6 unipolar, 3 bipolar) and 11 healthy low-risk twins without an affected co-twin (8 DZ, 3 MZ), mean age 32.4±5.0 years (range 25-40) were identified from a larger sample obtained by linking information from the Danish Twin Register and the Danish Psychiatric Central Register. To secure a homogenous and substantial risk-load in the high-risk group the following inclusion criteria were applied: 1) at onset of mood disorder, the co-twin was less than 35 yrs, and 2) the number of years passed from age of onset of the co-twin to inclusion of the unaffected twin should be less than 5 years.

The female:male ratio was 6:3 and 7:4 in the high and low-risk group, which reflects the gender frequency in depression. In the high-risk group, the average age of onset for affective disorder in the co-twin was 28.8±3.9 yrs, and the average of years passed from age of onset to inclusion of the unaffected twin was 3.5±1.2 yrs.

Clinical data, symptom scores, and personality assessment. Participants were interviewed by a trained clinician (MV) using semi-structured interviews. Diagnoses were made using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) version 2.1. All persons with a lifetime (current or past) diagnosis of affective disorder, schizoaffective disorder, or schizophrenia according to SCAN interview were excluded from the study. People with any significant brain disease were excluded. The Hamilton Depression Scale HAM-D, 17-item[31] was used to assess depressive symptoms. Further, self-rating of psychopathology was assessed with the 21-item Beck Depression Inventory (BDI 21)[32]. No significant differences were found in the prevalence of somatic diagnoses between risk groups and routine blood samples showed no differences between the groups. The assessment is described in detail in Christensen et al[33]. Participants were weighed and their heights were measured at the day of the PET-scan. Smoking status and weekly alcohol consumption based on the last two weeks were also registered. Education levels was scored from 1 to 5 according to Mortensen et al[34].

Since the personality trait neuroticism has been associated with risk for depression[35, 36] and neuroticism recently was found to correlate to frontolimbic serotonin 2A receptor binding[37] the participants also completed the Danish version of the 240 item

NEO-PI-R (NEO Personality Inventory Revised) self-report personality questionnaire[38] on the same day as the PET scanning. NEO-PI-R evaluates the broad personality dimensions of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness. Each dimension or factor score is derived by adding the scores from assessment of six constituent personality traits (facets) and each trait score is derived by adding the scores on 8 items in 0 – 4 Likert format[39].

Imaging: The SERT binding was imaged with [^{11}C]DASB PET based on 90 min dynamic acquisition starting immediately after bolus injection of 5.0±1.9 in the high-risk twins and 5.7±1.4 MBq [^{11}C]DASB/kg body weight in the low-risk twins; there was no significant difference in injected dose/kg. The acquisition consisted of 36 time frames, increasing progressively in duration from 10 sec to 10 min. The attenuation and decay corrected recordings were reconstructed by filtered back projection using a Hann filter (6mm). PET scans were performed with an 18-ring GE-Advance scanner (General Electric, Milwaukee, WI, USA), operating in 3D acquisition mode, producing 35 image slices with an interslice distance of 4.25 mm. To minimize movement during the scan the subjects had their heads lightly fixed with headbands.

Magnetic resonance imaging (MRI) was conducted on a Siemens Magnetom Trio 3T MR scanner with an eight-channel head coil (Invivo, FL, USA). High-resolution 3D T1-weighted, sagittal, magnetization prepared rapid gradient echo (MPRAGE) scans of the head (echo time (TE)/ repetition time (TR)/ inversion time (TI) = 3.93/1540/800 ms; flip angle= 9°; field of view (FOV) = 256 mm; matrix 256x256; 1x1x1mm voxels; 192 slices) and 2D T2-weighted, axial, Turbo Spin Echo (TSE) scans of the whole brain (TE1/TE2/TR= 17/100/9000 ms; flip angle = 150^{o}; FOV = 220 mm, matrix = 256x256) were acquired. Both T1 and T2 images were corrected for spatial distortions due to non-linearity in the gradient system of the scanner[40] using the Gradient Non-Linearity Distortion Correction software distributed by the Biomedical Informatics Research Network (http://www.nbirn.net). Subsequently, non-uniformity correction was performed with two iterations of the N3 program[41]. The resulting T1 images were intensity normalized to a mean value of 1000.

To enable extraction of the PET Volume of Interest (VOI)-signal from gray matter voxels only, MR images were segmented into gray matter, white matter, and

cerebrospinal fluid tissue classes. Tissue classification was performed with SPM2 (Welcome Department of Cognitive Neurology, University College London, UK) and the Hidden Markov Random Field (HMRF) model as implemented in the SPM2 VBM toolbox developed by Christian Gaser (University of Jena, Department of Psychiatry: http://dbm.neuro.uni-jena.de/vbm/). The T1 weighted template and associated a priori gray and white matter, and cerebrospinal fluid tissue maps that were used for tissue segmentation were created specifically for our imaging center and were based on high resolution MPRAGE scans of 185 healthy controls acquired on the Siemens Magnetom Trio 3T MR scanner (Mean age= 38.6, SD = 15.5, Median age = 30, age range = 18 – 82; 102 males; 83 females; 166 right handed, 18 left handed, 1 ambidexter). Finally, tissue probability images were cleaned for extra-cerebral tissue using an automatically created brain mask based on the gradient non-linearity corrected T2 image that was coregistered to the corresponding T1 image by a 6 degrees of freedom mutual information transformation.

Movement correction and co-registration: To correct for movements during the [^{11}C]DASB PET scan, all frames from 10 to 36 were aligned using AIR 5.2.5[42]. The frames acquired for the first 2 minutes did not contain enough information to be reliably aligned. Before alignment, each frame was filtered with a 12 mm Gaussian filter and thresholded at the 80% fractile of the voxel count values in the image. These parameters were chosen by visual inspection of tresholded images to ensure that they included brain gray matter voxels. The rigid transformation was estimated for each frame to a selected single frame with sufficient structural information (frame 26: 20-25 min post injection) using the scaled least squares cost-function in AIR. Subsequently, single frames were resliced and converted to a dynamic Analyze image file format.

The [^{11}C]DASB PET image (based on an average of frame 10 – 36) was co-registered to the MPRAGE image using the AIR algorithm[42]. The quality of each co-registration was evaluated by visual inspection in three planes.

Volumes of interest (VOIs) and partial volume correction. Based on the method by Svarer et al.[43] volumes of interest were automatically delineated on each individual's transaxial MRI slices in a strictly user-independent fashion. This method utilizes a

VOI probability map based on a template set of ten MRIs, where VOIs have been defined manually. Through the aligned MR images, the VOIs were transferred onto the PET images. The volumes of interest included the orbitofrontal cortex, the dorsolateral prefrontal cortex (DLPFC), the ventrolateral prefrontal cortex (VLPFC), anterior cingulate, caudaute, putamen, thalamus, and midbrain. DLPFC represents Brodmann area 9 and 46, VLPFC represents Brodmann area 44, 45, and 47. Those regions were delineated on the template set according to Petrides et al. [44]. A region representing the raphé nuclei was delineated according to Kalbitzer et al [45]. A volume representing the cerebellum was also defined in order to assess non-specific binding. For bilateral VOIs, left and right were averaged.

Quantification of SERT: The binding potential of specific tracer binding (BP_{ND}) was calculated using the Ichise Multi-linear reference Tissue Method 2 (MRTM2) with cerebellum as a reference region and fixing of k2' (clearance rate constant from cerebellum) by applying MRTM on time-activity curve data from a high binding region. The time-activity curve was composed from volume-weighted average of thalamus, putamen and caudate[46], except that midbrain was not included. Cerebellum (excluding cerebellar vermis) was used as a reference region, representing non-specific binding only[43, 47]. The kinetic modelling was performed using the PMOD software version 2.9, build 2 (PMOD Technologies).

Time-activity curves were extracted from gray matter voxels only, except for the midbrain and raphé nuclei, where the tissue structure does not allow for reliable MRI-separation of gray and white matter.

Statistics: Group comparisons of clinical parameters and symptom scores were performed by Mann-Whitney tests. Main effects of age, gender, BMI, smoking, BDI depression, Hamilton, and neuroticism on regional SERT-binding were tested by univariate linear regression. Effects of risk-status on regional SERT binding was tested in a multiple linear regression analysis adjusting for age (degrees of freedom:18) since an age effect on ^{11}C-DASB binding has been found in in vivo imaging studies including a larger age span than ours[24, 48, 49]. Effect sizes with 95% confidence limits, degrees of freedom, and two-tailed p-values are reported.

Results

Demographical data and symptom scores. As illustrated in table 1, the proportion of smokers in the high risk group was larger (p=0.002) but the difference in amount of tobacco previously consumed ("pack years"), was only borderline significant (p=0.06). Age, depression symptom scores and personality profiles of the high-risk and low-risk group were not statistically different.

Main effects of age, gender, BMI, smoking, depressive symptoms, and neuroticism scores on regional SERT-binding: In spite of the narrow age range (25.1-39.7 years), a tendency for a decrease in SERT binding with age was observed in anterior cingulate (p=0.076). Such an age-dependent decline has previously been found in larger samples with a wider age-range than ours[24, 48, 49]. In the remaining regions the p-values of negative correlations between SERT-binding and age ranged between 0.15 and 0.65. We saw no main effects of gender (0.97>p>0.19), BMI (0.94>p>0.34, smoking-status (0.94>p>0.10), BDI depression score (0.81>p>0.15), Hamilton (0.90>p>0.06), or neuroticism (0.77>p>0.17) in our sample. In one single region, the raphé nuclei, Hamilton score tended to be positively correlated with SERT-binding (p=0.06). However, this p-value was not corrected for multiple comparisons and was regarded as coincidental.

Effect of risk-status on regional SERT binding

As illustrated in table 2 and figure 1 the high-risk group had a lower SERT-binding in anterior cingulate and the DLPFC. The finding in DLPFC remained significant after a conservative Bonferroni correction for multiple comparisons of the 9 regions tested (p=0.016). In the putamen, there was a borderline significant effect of risk. There was no effect of risk-status in orbitofrontal cortex, thalamus, caudatus, midbrain, and raphé nuclei. Age, as a covariate in the analysis of risk-status effects, had a significant or borderline significant negative effect on binding, DLPFC: p=0.072 and anterior cingulate: p=0.039.

Post hoc, when the analysis was carried out with adjustment for both age and the Hamilton score, it was confirmed that the Hamilton score did not significantly

contribute to the model in any region ($p>0.64$) nor did it change the effect of risk-status in DLPFC or anterior cingulate.

Discussion

We show that familial predisposition to mood disorder, in the absence of depressive symptoms, is associated with a low SERT binding in DLPFC and anterior cingulate. The observed reduction in SERT binding in DLPFC persisted after a conservative correction for multiple comparisons.

Although the sample size included in our study was modest, the twin design assured a maximal contrast and thereby a gain in statistical power. We also assessed SERT-binding in frontal brain regions where the SERT density is low to moderate. However, test-retest [^{11}C]-DASB data have shown that in frontal regions the variability of BP_{ND} derived from the MRTM2 method is 8.7%[50]. This is substantially lower than the differences of 15% and 35% we found in the anterior cingulate and DLPFC and therefore, we regard the findings as robust.

We studied twins and, thus, the outcome may not be representative of singletons. However, several studies support that mood disorders in twins are typical to that of the general population; both monozygotic and dizygotic twins resemble the background population in their risk for mood disorders[51, 52], and in their level and variability of self-reported psychiatric symptoms[53]. It should be emphasized that the study was not designed as a traditional twin study, to answer questions about the relative influence of genetic versus early environmental factors. Rather, our aim was to identify healthy subjects at high or low risk for mood disorders, and the twins (even the DZ) served that purpose better than other relatives to patients with mood disorders.

In the high-risk group the fraction of monozygotic twins was 44%, mean age of onset was < 30 years, and the average number of years passed from age of onset of the co-twin' affective disorder to inclusion of the healthy twin was 3.5 years. This means that the high-risk group still is at substantial risk of development of a mood disorder[1]. In the low-risk group the fraction of monozygotic twins was 27%, adding to the contrast in genetic risk.

The fraction of predisposition through bipolar co-twins was high in our sample: 33% (6 unipolar and 3 bipolar). It is possible that unipolar and bipolar

disorder constitute a continuous spectrum, rather than being categorically distinct[54]. More importantly, keeping our main goal in mind, there is no difference in the frequency of unipolar depression in relatives of unipolars and bipolars, leaving the risk unaltered in the healthy at-risk twin group[55].

To our knowledge this is the first study to investigate SERT binding as a trait characteristic of mood disorders. Interestingly, a large postmortem study in brains from non-suicide, antidepressant-free subjects with a *history* of life-time depression showed a reduction of SERT, particularly, in DLPFC[56]. Also, reductions of serotonergic axons[57] and neuronal density[58] were observed in DLPFC in postmortem studies of depression. Accordingly, several reports based on different techniques place serotonergic neurotransmission in DLPFC as a key factor in depression. Further, functional neuroimaging studies strongly support the involvement of DLPFC in depression based on emotional and cognitive activation paradigms and studies of treatment effects, as reviewed by Fitzgerald et al[59]. The anterior cingulate is also key in the pathophysiology of depression. Metabolic[60-62], structural[63], and serotonin related trait abnormalities[64] have been identified in this particular region. We therefore suggest that a reduction in SERT binding in DLPFC and possibly anterior cingulate is a trait marker of depression.

Low DLPFC SERT-binding could be caused by different mechanisms or a combination of these, but our data do not allow for a distinction between these explanations. These include: 1) lower extracellular serotonin levels facilitating SERT internalization and degradation[65], 2) reduced number of serotonergic projections to DLPFC and anterior cingulate, 3) decreased SERT expression determined by genetic and/or early environmental factors, and/or 4) higher endogenous serotonin levels competing with [^{11}C]DASB at the binding site. This latter explanation is considered less likely, since [^{11}C]DASB binding is relatively insensitive to acute modulations of endogenous levels of serotonin[66, 67].

Since we did not observe significant reductions in SERT in midbrain and raphé nuclei, regions primarily representing serotonergic neuronal cell bodies, we suggest that the lower SERT-binding found in DLPFC and anterior cingulate reflects modulations of SERT located in serotonergic nerve terminals rather than selective loss of serotonergic neurons. In a postmortem study it was found that serotonergic neurons in the dorsal raphe nucleus express 54% lower amounts of mRNA in brains

from depressed suicide patients[68]. However, decreased amounts of mRNA does not necessarily lead to lower SERT protein levels as it may be associated with a low turnover of SERT. The finding that a functional polymorphism of the rate limiting enzyme of neuronal serotonin synthesis was more prevalent in subjects with a history of unipolar depression suggests that reductions in serotonin synthesis and levels may be a genetically based risk factor for developing unipolar depression[69].

The lower DLPFC SERT binding may be caused by a primary genetic effect on the SERT. In support of this, SERT knock-out mice show depressive-like behavior and also have a reduced serotonergic cell number and firing rate in the dorsal raphe nuclei[70]. Of note, the low expressing ”s” variant of the SERT promoter gene, a risk factor for depression in combination with environmental stress[9, 71], had a similar frequency in our high- and low-risk groups.

We speculate that the reduced DLPFC SERT binding in high-risk subjects, observed in this study may represent compensatory adaptations to inherited dysfunctions in serotonin homeostasis. Only when such compensatory mechanisms break down, depression occurs. Indeed, recent [^{11}C]DASB-PET studies have suggested that SERT binding in several cortical regions is elevated during depressive episodes in mood disorders[28], and that the severity of depressive symptoms correlate positively with SERT-binding[25]. In a different study where [11C]McN5652 was used, decreased SERT-binding was found in the anterior cingulate of depressed patients with bipolar disorder[20]. The latter study was, however, potentially confounded by treatment effects.

In transfected human embryonic kidney cell lines the cell surface located SERT density respond to extracellular serotonin levels[65], but it is unknown if such mechanisms operate in the brain of healthy people, in response to acute and short-term decrease in synaptic serotonin levels[66, 67]. Down-regulation of SERT may require sustained exposure to low levels of serotonin. However, internalization of SERT in response to low levels of extracellular serotonin without a concomitant degradation would not be registered by [^{11}C]DASB-PET, since this methodology most probably does not allow for a distinction between SERT proteins on the cell surface or within the cell.

In our data, SERT-binding tended to decrease with age in DLPFC and anterior cingulate, even with the relatively small range. This supports that age needs to be considered in SERT studies, in line with some[24, 48], although not all imaging studies[25]. No significant gender effects were observed in our data. In larger samples, however, lower SERT-binding in women with a history of depression are reported for several frontal and subcortical regions[56], a finding that may be related to the higher rate of depression in women, and/or gender differences in genetic vulnerability to environmental stress[72, 73]. Meyer et al have shown that SERT-binding correlates with dysfunctional attitudes or negativistic thinking in depressed patients[25]. We did not see a correlation between SERT binding and depressive symptoms in our healthy subjects but as expected, the subjects only had very few symptoms and the matter cannot be validly assessed.

The predisposed group smoked more than the controls. This is well in line with the observation that individuals with current or past depression are more likely to have been smokers at some point in their lives, and it may even reflect common genetic factors between smoking addiction and risk of developing depression[74]. In theory, nicotine could lead to alterations in SERT-binding through adaptation to increased central serotonin release. However, no SERT-binding effect of smoking on the regions most likely to be influenced, striatum or diencephalon was observed in a large scale study of 42 healthy subjects[75].

In conclusion, we find a lower SERT binding in the dorsolateral prefrontal cortex and anterior cingulate in healthy subjects at high familial risk of developing mood disorder. The reduction in SERT binding may represent a trait marker for mood disorder established either during early brain development, through a reduced number of serotonergic projections, and/or compensatory adaptations to low synaptic serotonin.

ACKNOWLEDGEMENTS

We are grateful to the participants for their willingness to participate. We wish to thank Karin Stahr, Dorthe Givard and the staff at the PET centre, Rigshospitalet for their superb technical assistance. Klaus Kähler Holst is thanked for expert statistical assistance. Thanks to the Department of Psychiatric Demography, University of Aarhus, Psychiatric Hospital, Riskov, Denmark, and to the Danish Twin Registry for cooperation in the study.

The John and Birthe Meyer Foundation is thanked for the donation of the Cyclotron and PET scanner. This work was generously supported by Danish Medical Research Council, The Health Science Faculty, University of Copenhagen, the 1991 Pharmacy Foundation, The Lundbeck Foundation, the EU 6th framework programme (EC-FP6-project DiMI (LSHB-CT-2005-512146), Sawmill owner Jeppe Juhl and Wife Ovita Juhls Foundation, “Laegernes forsikringsforening af 1891”, and “Fonden af 1870”.

COMPETING INTEREST STATEMENT

The authors declare no competing financial interest.

Table 1: Clinical data and symptom scores

	High-risk N=9	*Low-risk N=11*	*p-value*
Age (yrs)	32.6 (26.6-38.2)	32.9 (25.1-39.7)	NS
Body mass index (kg/m^2)	24.6 (19.5-30.4)	26.0 (23.1-29.1)	NS
Alcohol (units/week)	2 (0-10)	4 (1-20)	NS
Proportion of smokers	67%	0%	p=0.002
History of tobacco use*	12 (0-20)	0 (0-12)	NS
Education score	3 (1-5)	4 (1-5)	NS
BDI_depression	0 (0-6)	0 (0-7)	NS
Hamilton	1.5 (0-5)	2 (0-5)	NS
Neuroticism	61 (53-77)	55 (35-118)	NS
ss or sl SERT-promoter allele	82%	78%	NS

Data are shown as medians with range in brackets. Group differences were not significant (Mann-Whitney). *Tobacco use in ”pack-years”: number of years of smoking 20 cigarettes per day. BDI: Becks depression inventory. BDI_depression: depression component (21 items). Hamilton: Hamilton Depression rating Scale score of depressive symptoms. Neuroticism: Neuroticism score from the 240 item NEO-PI-R questionnaire, Danish version.

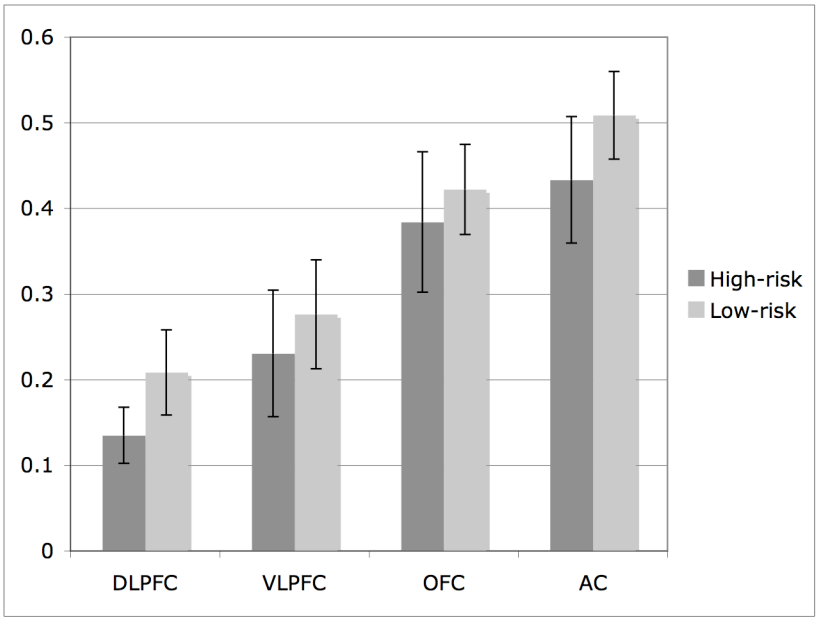
Table 2: Effect of risk-status on regional SERT binding

	Difference (HR-LR) BP_{ND}	*95% Confidence limits* BP_{ND}	*p-value*
DLPFC	-0.074	(-0.12 - -0.032)	0.0018
	(-35 %)	(-15 – -55%)	
VLPFC	-0.046	(-0.11- 0.02)	0.17
Orbitofrontal	-0.038	(-0.10 - 0.028)	0.24
Anterior cingulate	-0.075	(-0.14 - -0.014)	0.018
	(-15%)	(-3 – -26%)	
Thalamus	-0.048	(-0.32 - 0.23)	0.71
Caudate	-0.11	(-0.31- 0.10)	0.30
Putamen	-0.17	(-0.34 - 0.01)	0.06
Midbrain	-0.14	(-0.35 - 0.059)	0.15
Raphé nuclei	-0.23	(-0.61- 0.14)	0.20

Effects of risk-status evaluated in multiple linear regression analysis adjusting for age. DF: 17. DLPFC, dorsolateral prefrontal cortex; VLPFC: Ventrolateral prefrontal cortex. The differences between high-risk and low-risk age adjusted SERT binding (BP_{ND}) in percentages of low-risk age adjusted BP_{ND} are displayed in brackets. BP_{ND} was adjusted to that expected of a 30 year old.

Figure 1. Age-adjusted SERT binding (BP_{ND}) in various brain regions in high-risk versus low-risk twins. Panel A: Cortical regions. Panel B: Subcortical regions. Data are shown as mean and error bars indicate standard deviations. BP_{ND} was adjusted to that expected of a 30 year old.

A



B

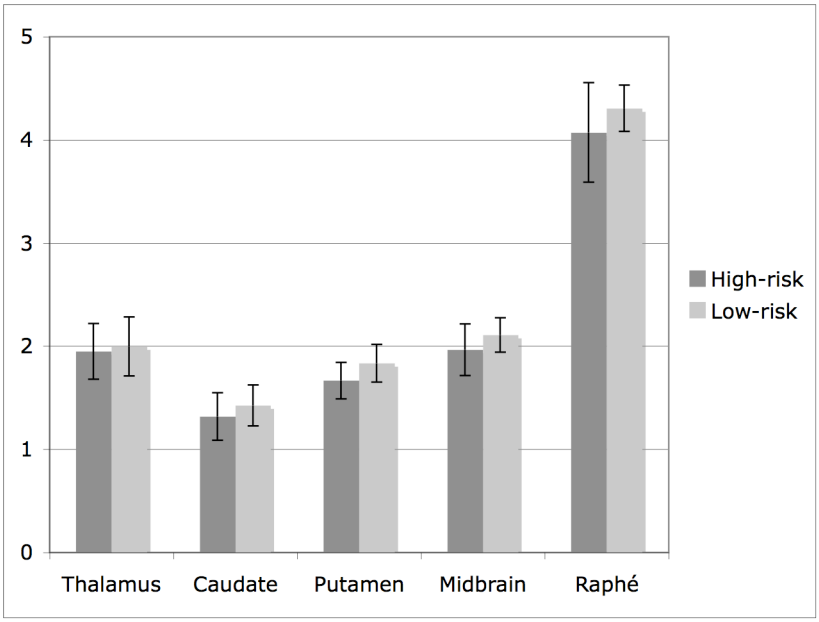
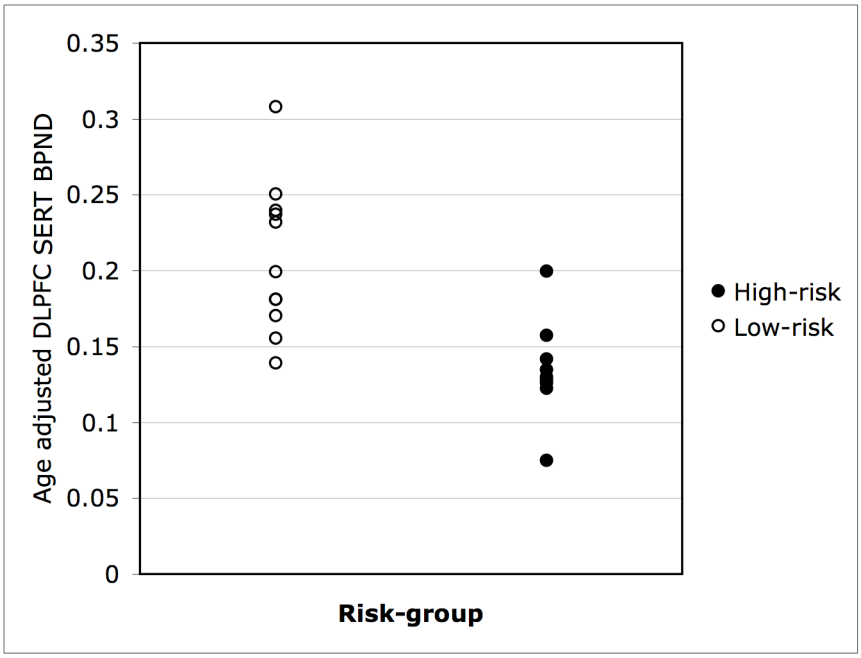
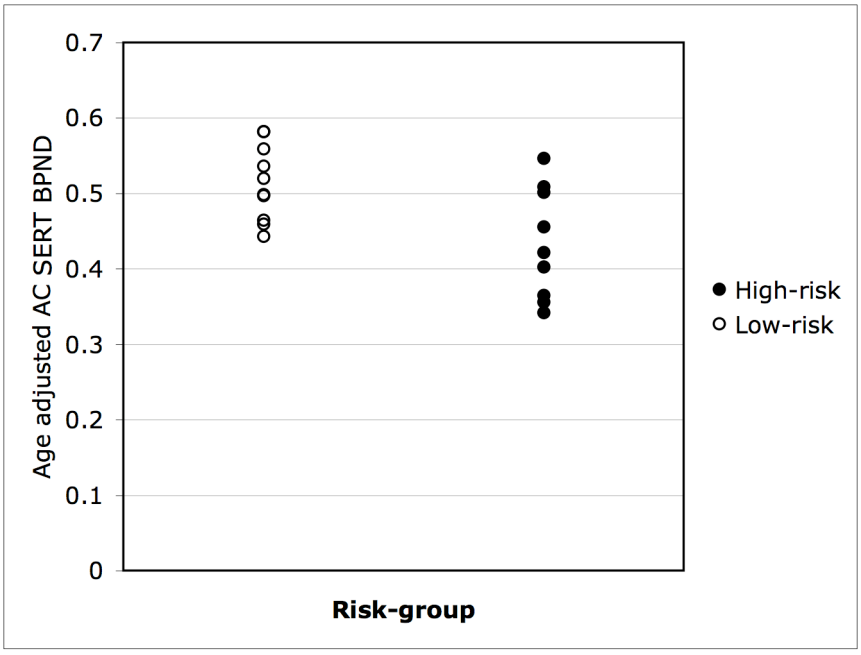


Figure 2. Effect of familial risk of mood disorder on age-adjusted regional SERT-binding (BP_{ND}). High-risk subjects had a lower binding in DLPFC (bilateral dorsolateral prefrontal cortex), p=0.0018 (panel A), and anterior cingulate, p=0.018 (panel B). BP_{ND} was adjusted to represent a 30-year old person.

A



B



References

1. Lyons MJ, Eisen SA, Goldberg J, et al. A registry-based twin study of depression in men. *Arch Gen Psychiatry.* May 1998;55(5):468-472.
2. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. The lifetime history of major depression in women. Reliability of diagnosis and heritability. *Arch Gen Psychiatry.* Nov 1993;50(11):863-870.
3. Kendler KS, Gardner CO, Prescott CA. Clinical characteristics of major depression that predict risk of depression in relatives. *Arch Gen Psychiatry.* Apr 1999;56(4):322-327.
4. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry.* Oct 2000;157(10):1552-1562.
5. McGuffin P, Katz R, Rutherford J. Nature, nurture and depression: a twin study. *Psychol Med.* May 1991;21(2):329-335.
6. Kendler KS, Gatz M, Gardner CO, Pedersen NL. A Swedish national twin study of lifetime major depression. *Am J Psychiatry.* Jan 2006;163(1):109-114.
7. Levinson DF. The genetics of depression: a review. *Biol Psychiatry.* Jul 15 2006;60(2):84-92.
8. Holmans P, Weissman MM, Zubenko GS, et al. Genetics of recurrent early-onset major depression (GenRED): final genome scan report. *Am J Psychiatry.* Feb 2007;164(2):248-258.
9. Caspi A, Sugden K, Moffitt TE, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science.* Jul 18 2003;301(5631):386-389.
10. Praschak-Rieder N, Kennedy J, Wilson AA, et al. Novel 5-HTTLPR Allele Associates with Higher Serotonin Transporter Binding in Putamen: A [(11)C] DASB Positron Emission Tomography Study. *Biol Psychiatry.* Jan 6 2007.
11. Shioe K, Ichimiya T, Suhara T, et al. No association between genotype of the promoter region of serotonin transporter gene and serotonin transporter binding in human brain measured by PET. *Synapse.* Jun 15 2003;48(4):184-188.
12. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry.* Jun 15 2001;49(12):1023-1039.
13. Kajantie E. Fetal origins of stress-related adult disease. *Ann N Y Acad Sci.* Nov 2006;1083:11-27.
14. Talge NM, Neal C, Glover V. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry.* Mar-Apr 2007;48(3-4):245-261.
15. Gaspar P, Cases O, Maroteaux L. The developmental role of serotonin: news from mouse molecular genetics. *Nat Rev Neurosci.* Dec 2003;4(12):1002-1012.
16. Taylor MJ, Freemantle N, Geddes JR, Bhagwagar Z. Early onset of selective serotonin reuptake inhibitor antidepressant action: systematic review and meta-analysis. *Arch Gen Psychiatry.* Nov 2006;63(11):1217-1223.
17. Klaassen T, Riedel WJ, van Someren A, Deutz NE, Honig A, van Praag HM. Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. *Biol Psychiatry.* Aug 15 1999;46(4):489-497.
18. Quintin P, Benkelfat C, Launay JM, et al. Clinical and neurochemical effect of acute tryptophan depletion in unaffected relatives of patients with bipolar affective disorder. *Biol Psychiatry.* Aug 1 2001;50(3):184-190.
19. Stockmeier CA. Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. *J Psychiatr Res.* Sep-Oct 2003;37(5):357-373.
20. Oquendo MA, Hastings RS, Huang YY, et al. Brain serotonin transporter binding in depressed patients with bipolar disorder using positron emission tomography. *Arch Gen Psychiatry.* Feb 2007;64(2):201-208.
21. Parsey RV, Hastings RS, Oquendo MA, et al. Lower serotonin transporter binding potential in the human brain during major depressive episodes. *Am J Psychiatry.* Jan 2006;163(1):52-58.
22. Malison RT, Price LH, Berman R, et al. 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.* Dec 1 1998;44(11):1090-1098.
23. Willeit M, Praschak-Rieder N, Neumeister A, et al. [123I]-beta-CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. *Biol Psychiatry.* Mar 15 2000;47(6):482-489.
24. Meyer JH, Wilson AA, Ginovart N, et al. Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [(11)C]DASB PET imaging study. *Am J Psychiatry.* Nov 2001;158(11):1843-1849.

25. Meyer JH, Houle S, Sagrati S, et al. Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry.* Dec 2004;61(12):1271-1279.

26. Dahlstrom M, Ahonen A, Ebeling H, Torniainen P, Heikkila J, Moilanen I. Elevated hypothalamic/midbrain serotonin (monoamine) transporter availability in depressive drug-naive children and adolescents. *Mol Psychiatry.* Sep 2000;5(5):514-522.

27. Ichimiya T, Suhara T, Sudo Y, et al. Serotonin transporter binding in patients with mood disorders: a PET study with [11C](+)McN5652. *Biol Psychiatry.* May 1 2002;51(9):715-722.

28. Cannon DM, Ichise M, Fromm SJ, et al. Serotonin transporter binding in bipolar disorder assessed using [11C]DASB and positron emission tomography. *Biol Psychiatry.* Aug 1 2006;60(3):207-217.

29. Cannon DM, Ichise M, Rollis D, et al. Elevated Serotonin Transporter Binding in Major Depressive Disorder Assessed Using Positron Emission Tomography and [(11)C]DASB; Comparison with Bipolar Disorder. *Biol Psychiatry.* Aug 1 2007.

30. Meyer JH. Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. *J Psychiatry Neurosci.* Mar 2007;32(2):86-102.

31. Hamilton M. Development of a rating scale for primary depressive illness. *Br J Soc Clin Psychol.* Dec 1967;6(4):278-296.

32. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry.* Jun 1961;4:561-571.

33. Christensen MV, Kyvik KO, Kessing LV. Subclinical psychopathology and socio-economic status in unaffected twins discordant for affective disorder. *J Psychiatr Res.* Apr-Jun 2007;41(3-4):229-238.

34. Mortensen E, Gade A. On the relation between demographic variables and neuropsychological test performance. *Scand J Psychol.* 1993;34: 305-317.

35. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. A longitudinal twin study of personality and major depression in women. *Arch Gen Psychiatry.* Nov 1993;50(11):853-862.

36. Fanous AH, Neale MC, Aggen SH, Kendler KS. A longitudinal study of personality and major depression in a population-based sample of male twins. *Psychol Med.* Aug 2007;37(8):1163-1172.

37. Frokjaer VG, Mortensen EL, Nielsen FA, et al. Frontolimbic Serotonin 2A Receptor Binding in Healthy Subjects Is Associated with Personality Risk Factors for Affective Disorder. *Biol Psychiatry.* Sep 18 2007.

38. Skovdahl-Hansen H, Mortensen, EL., Scioetz, HK. *Dokumentation for den danske udgave af NEO PI-R og NEO PI-R Kort Version.* Copenhagen, Denmark: Dansk psykologisk forlag; 2004.

39. Costa PT, McCrae RR. *Professional manual for Revised NEO Personality Inventory*: Odessa FL: Psychological Assessment Resources; 1992.

40. Jovicich J, Czanner S, Greve D, et al. Reliability in multi-site structural MRI studies: effects of gradient non-linearity correction on phantom and human data. *Neuroimage.* Apr 1 2006;30(2):436-443.

41. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging.* Feb 1998;17(1):87-97.

42. Woods RP, Cherry SR, Mazziotta JC. Rapid automated algorithm for aligning and reslicing PET images. *J Comput Assist Tomogr.* Jul-Aug 1992;16(4):620-633.

43. Svarer C, Madsen K, Hasselbalch SG, et al. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage.* Feb 15 2005;24(4):969-979.

44. Petrides M. Lateral prefrontal cortex: architectonic and functional organization. *Philos Trans R Soc Lond B Biol Sci.* Apr 29 2005;360(1456):781-795.

45. Kalbitzer J, Svarer C, Frokjaer V, et al. Evaluating a new observer-independent approach to in-vivo analysis of 5-hydroxytryptamine transporter activity in human raphe nuclei using positron emission tomography. *Eur J Nucl Med Mol Imaging.* 2007;34, supp.2:205.

46. Ichise M, Liow JS, Lu JQ, et al. Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J Cereb Blood Flow Metab.* Sep 2003;23(9):1096-1112.

47. Kish SJ, Furukawa Y, Chang LJ, et al. Regional distribution of serotonin transporter protein in postmortem human brain: is the cerebellum a SERT-free brain region? *Nucl Med Biol.* Feb 2005;32(2):123-128.

48. Pirker W, Asenbaum S, Hauk M, et al. Imaging serotonin and dopamine transporters with 123I-beta-CIT SPECT: binding kinetics and effects of normal aging. *J Nucl Med.* Jan 2000;41(1):36-44.

49. van Dyck CH, Malison RT, Seibyl JP, et al. Age-related decline in central serotonin transporter availability with [(123)I]beta-CIT SPECT. *Neurobiol Aging.* Jul-Aug 2000;21(4):497-501.

50. Kim JS, Ichise M, Sangare J, Innis RB. PET imaging of serotonin transporters with [11C]DASB: test-retest reproducibility using a multilinear reference tissue parametric imaging method. *J Nucl Med.* Feb 2006;47(2):208-214.

51. Kendler KS, Pedersen NL, Farahmand BY, Persson PG. The treated incidence of psychotic and affective illness in twins compared with population expectation: a study in the Swedish Twin and Psychiatric Registries. *Psychol Med.* Nov 1996;26(6):1135-1144.

52. Klaning U, Laursen TM, Licht RW, Kyvik KO, Skytthe A, Mortensen PB. Is the risk of bipolar disorder in twins equal to the risk in singletons? A nationwide register-based study. *J Affect Disord.* Aug 2004;81(2):141-145.

53. Kendler KS, Martin NG, Heath AC, Eaves LJ. Self-report psychiatric symptoms in twins and their nontwin relatives: are twins different? *Am J Med Genet.* Dec 18 1995;60(6):588-591.

54. Akiskal HS, Benazzi F. The DSM-IV and ICD-10 categories of recurrent [major] depressive and bipolar II disorders: evidence that they lie on a dimensional spectrum. *J Affect Disord.* May 2006;92(1):45-54.

55. Kutcher S, Marton P. Affective disorders in first-degree relatives of adolescent onset bipolars, unipolars, and normal controls. *J Am Acad Child Adolesc Psychiatry.* Jan 1991;30(1):75-78.

56. Mann JJ, Huang YY, Underwood MD, et al. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. *Arch Gen Psychiatry.* Aug 2000;57(8):729-738.

57. Austin MC, Whitehead RE, Edgar CL, Janosky JE, Lewis DA. Localized decrease in serotonin transporter-immunoreactive axons in the prefrontal cortex of depressed subjects committing suicide. *Neuroscience.* 2002;114(3):807-815.

58. Rajkowska G, Miguel-Hidalgo JJ, Wei J, et al. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry.* May 1 1999;45(9):1085-1098.

59. Fitzgerald PB, Oxley TJ, Laird AR, Kulkarni J, Egan GF, Daskalakis ZJ. An analysis of functional neuroimaging studies of dorsolateral prefrontal cortical activity in depression. *Psychiatry Res.* Nov 22 2006;148(1):33-45.

60. Mayberg HS, Brannan SK, Mahurin RK, et al. Cingulate function in depression: a potential predictor of treatment response. *Neuroreport.* Mar 3 1997;8(4):1057-1061.

61. Drevets WC, Price JL, Simpson JR, Jr., et al. Subgenual prefrontal cortex abnormalities in mood disorders. *Nature.* Apr 24 1997;386(6627):824-827.

62. Videbech P. PET measurements of brain glucose metabolism and blood flow in major depressive disorder: a critical review. *Acta Psychiatr Scand.* Jan 2000;101(1):11-20.

63. Sheline YI. Neuroimaging studies of mood disorder effects on the brain. *Biol Psychiatry.* Aug 1 2003;54(3):338-352.

64. Neumeister A, Nugent AC, Waldeck T, et al. Neural and behavioral responses to tryptophan depletion in unmedicated patients with remitted major depressive disorder and controls. *Arch Gen Psychiatry.* Aug 2004;61(8):765-773.

65. Ramamoorthy S, Blakely RD. Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. *Science.* Jul 30 1999;285(5428):763-766.

66. Talbot PS, Frankle WG, Hwang DR, et al. Effects of reduced endogenous 5-HT on the in vivo binding of the serotonin transporter radioligand 11C-DASB in healthy humans. *Synapse.* Mar 1 2005;55(3):164-175.

67. Praschak-Rieder N, Wilson AA, Hussey D, et al. Effects of tryptophan depletion on the serotonin transporter in healthy humans. *Biol Psychiatry.* Nov 15 2005;58(10):825-830.

68. Arango V, Underwood MD, Boldrini M, et al. Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology.* Dec 2001;25(6):892-903.

69. Zhang X, Gainetdinov RR, Beaulieu JM, et al. Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron.* Jan 6 2005;45(1):11-16.

70. Lira A, Zhou M, Castanon N, et al. Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. *Biol Psychiatry.* Nov 15 2003;54(10):960-971.

71. Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch Gen Psychiatry.* May 2005;62(5):529-535.

72. Brummett BH, Boyle SH, Siegler IC, et al. Effects of Environmental Stress and Gender on Associations among Symptoms of Depression and the Serotonin Transporter Gene Linked Polymorphic Region (5-HTTLPR). *Behav Genet.* Oct 23 2007.

73. Barr CS, Newman TK, Schwandt M, et al. Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. *Proc Natl Acad Sci U S A*. Aug 17 2004;101(33):12358-12363.

74. Kendler KS, Neale MC, MacLean CJ, Heath AC, Eaves LJ, Kessler RC. Smoking and major depression. A causal analysis. *Arch Gen Psychiatry*. Jan 1993;50(1):36-43.

75. Staley JK, Krishnan-Sarin S, Zoghbi S, et al. Sex differences in [123I]beta-CIT SPECT measures of dopamine and serotonin transporter availability in healthy smokers and nonsmokers. *Synapse.* Sep 15 2001;41(4):275-284.