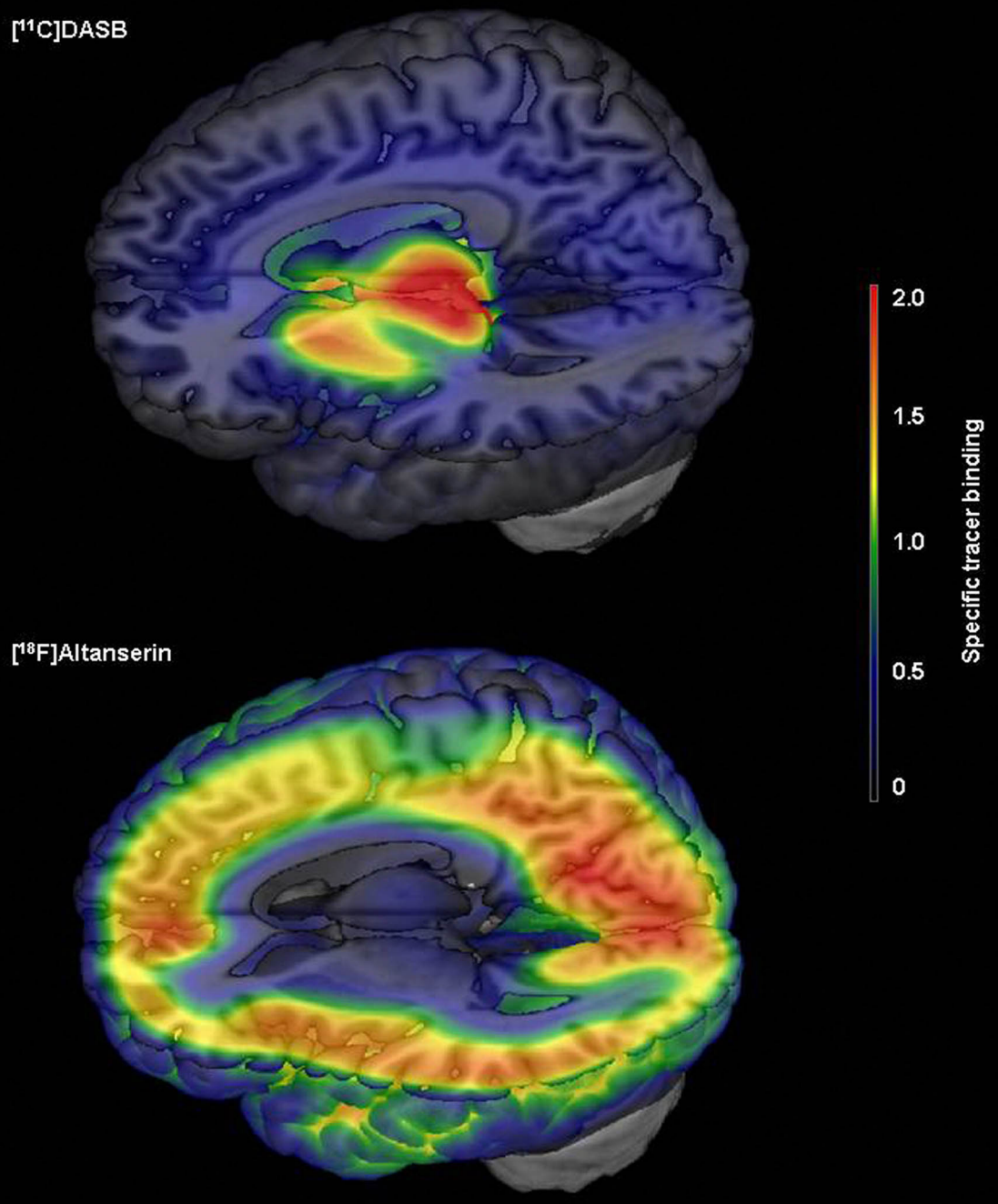
In Vivo Serotonergic Markers in Overweight and Schizophrenic Human Subjects



Ph.D. Thesis

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David Erritzøe, Copenhagen, 2008

Project summary

This Ph.D.-project focuses on 1) the cerebral serotonin 2A (5-HT_{2A}) receptor and the serotonin transporter (SERT) in relation to overweight/obesity, tobacco smoking and alcohol consumption, and 2) the cerebral 5-HT_{2A} receptor in relation to schizophrenia.

Manipulations of the serotonin (5-hydroxytryptamine, 5-HT) levels in the brain can induce impulsive behavior and influence our reactivity to conditioned reinforcers. Eating behavior, tobacco smoking, and alcohol consumption are all known to be related to altered serotonergic neurotransmission; thus serotonergic hypofunction leads to increased food and alcohol intake whereas stimulation of the serotonergic system induces weight reduction and decreased food/alcohol intake as well as tobacco smoking.

5-HT_{2A} receptor stimulating compounds such as lysergic acid diethylamide (LSD) induce hallucinogenic symptoms that are similar to schizophrenic symptoms, whereas atypical antipsychotics have antagonistic effects on the 5-HT_{2A} receptors, both pointing at a specific role of the 5-HT_{2A} receptor in the pathophysiology of schizophrenia. A significant decrease in the distribution of this receptor, especially in the frontal cortical regions, has been reported in several post mortem studies of schizophrenic patients whereas no significant differences in 5-HT_{2A} binding between neuroleptic-naive schizophrenic patients and healthy controls were found in two small PET studies

In vivo imaging techniques such as positron emission tomography (PET) have enabled the investigation of molecular markers of the cerebral transmitter systems. For example, the use of PET and the radioligands ^{18}F-altanserin and ^{11}C-DASB, allow for quantification of 5-HT_{2A} and the SERT, respectively, in the living human brain.

Using these techniques in two overlapping cohorts of human subjects - including both normal weighted, overweight and obese persons – it was investigated whether body mass index (BMI) was associated to the cerebral 5-HT_{2A} receptor and SERT binding. Further, the degree of alcohol consumption and tobacco smoking was related to these two brain 5-HT markers. The 5-HT_{2A} receptor binding was assessed in 136 healthy adult subjects and SERT binding was measured in 60 subjects. The primary regions of interest included a global neortical region consisting of a volume-weighted average of 8 cortical regions (for both markers), a high-binding subcortical region (consisting of the caudate nucleus, putamen, and thalamus), and midbrain (for SERT binding only). In a separate study of 15 neuroleptic-naive schizophrenic patients, cerebral 5-HT_{2A} binding in was

compared to 15 age-, gender- and BMI-matched healthy controls.

Using linear regression analysis, cortical 5-HT_{2A} binding was significantly positively correlated to BMI, whereas a significant negative correlation between BMI and cortical and subcortical SERT binding was detected. No significant associations were detected between alcohol or tobacco use and the binding of any of the two 5-HT markers. In comparison to healthy control subjects, cortical 5-HT_{2A} receptor binding was unchanged in schizophrenic patients but increased in the caudate nucleus.

We propose that the observation of globally decreased SERT and increased 5-HT_{2A} receptor binding in the brain is secondary to – and thereby a surrogate marker of - low 5-HT levels. Low synaptic 5-HT levels could lead both to a compensatory upregulation of cerebral 5-HT_{2A} receptor binding and to a downregulation of SERT. Also, hypofunction of serotonergic neurotransmission results in increased appetite and food intake and thereby to overweight/obesity. Thus, our observations support the important role of the serotonin transmitter system in regulation of body weight.

Dansk resume

Ph.D.-projektet omhandler ændringer i hjernens serotonin 2A ($5\text{-}HT_{2A}$) receptor og serotonintransporter (SERT) binding hos raske, overvægtige forsøgspersoner og hos neuroleptika-naïve, debuterende skizofrene patienter.

Fødeindtag, tobaksrygning og alkoholindtag er alle relateret til en ændret serotonerg neurotransmission; således fører en nedsat serotonerg tonus til et øget føde- og alkoholindtag, mens en stimulering af serotoninsystemet medfører nedsat fødeindtag og vægttab.

Omend de patofysiologiske overvejelser indenfor skizofrenisygdommen hidtil mest har fokuseret på forstyrrelser i dopamin- og glutamatsystemet, er der også stærke holdepunkter for involvering af $5\text{-}HT_{2A}$ receptoren. For eksempel inducerer $5\text{-}HT_{2A}$ receptor-stimulation med LSD hallucinationer af samme karakter som ved skizofren psykose og atypiske antipsykotika udviser $5\text{-}HT_{2A}$ receptorantagonisme. Ved en del post-mortem undersøgelser af hjernen hos skizofrene patienter har man da også kunne påvise nedsat $5\text{-}HT_{2A}$ receptorbinding, særlig i frontal kortex. In vivo undersøgelser med billeddannende metoder har derimod hidtil ikke kunne identificere sådanne forandringer hos nydiagnosticerede skizofrene.

Med billeddannende teknikker såsom positron emissionstomografi (PET) er det blevet muligt at visualisere og dermed kortlægge molekylære markører i hjernen hos levende mennesker. I denne PhD-afhandling anvendes de radioaktive sporstoffer ^{18}F-altanserin og ^{11}C-DASB til kvantificering af henholdsvis $5\text{-}HT_{2A}$ receptoren og serotonintransporteren (SERT), og i to overlappende grupper af forsøgspersoner blev det undersøgt om body mass index (BMI) var relateret til hjernens $5\text{-}HT_{2A}$ receptor og SERT binding. Forsøgspersonerne bestod af såvel normalvægtige som overvægtige og fede, men i øvrigt raske personer. Desuden blev omfanget af tobaksrygning og alkoholindtag sammenholdt med reguleringen af disse to serotonerge markører. $5\text{-}HT_{2A}$ receptor bindingen blev målt hos 136 raske voksne forsøgspersoner, mens SERT bindingen måltes hos 60 forsøgspersoner, hvoraf de 56 også indgik i den første gruppe. Der blev også foretaget en sammenligning af $5\text{-}HT_{2A}$ receptor og SERT bindingen hos de 56 personer, der fik foretaget begge PET skanninger.

I en lineær regressionsmodel var den kortikale $5\text{-}HT_{2A}$ binding signifikant positivt korreleret til BMI, mens der var en signifikant negativ korrelation mellem BMI og såvel

kortikal som subkortikal SERT binding. Der var ingen relation mellem hverken tobaksrygning eller alkoholindtag og niveauet af de to serotonerge markører. Sammenlignet med raske, alders-matchede forsøgspersoner var den kortikale 5-HT_{2A} receptor binding uændret hos de skizofrene patienter, som dog udviste forøget binding i nucleus caudatus. Der påvistes en omvendt U-formet relation mellem den præ- og den postsynaptiske serotonerge markør, hvilket kan forklares udfra en model, hvor hjernens serotonin niveau regulerer begge markører.

På baggrund af disse resultater fortolkes reduktionen i SERT og forøgelsen i 5-HT_{2A} receptor binding i hjernen hos overvægtige som værende sekundær til abnormt lavt niveau af serotonin i hjernen. Denne fortolkning underbygges af kendskabet til at nedsat serotonerg tonus medfører øget appetit og fødeindtag og dermed til overvægt/fedme.

List of papers

1. Erritzoe D., Frokjaer V.G., Haugbol S., Marner L., Svarer C., Holst K., Baaré W.F.C., Rasmussen P.M., Madsen J., Paulson O.B., Knudsen G.M. **Brain Serotonin 2A Receptor Binding: Relations to Body Mass Index, Tobacco and Alcohol Use.** *NeuroImage (2009) 46, 23-30*

2. Erritzoe D., Frokjaer V.G., Haarh M., Kalbitzer J., Svarer C., Holst K. K., Hansen D. L., Jernigan T., Lehel S., Knudsen G.M. **Cerebral Serotonin Transporter Binding is inversely related to Body Mass Index.** *(Manuscript submitted to NeuroImage, March 2009)*

3. Erritzoe D., Rasmussen H., Kristiansen K.T., Frokjaer V.G., Haugbol S., Pinborg L., Baaré W., Svarer C., Madsen J., Lublin H., Knudsen G.M., Glenthoj B.Y. **Cortical and Subcortical 5-HT_{2A} Receptor Binding in Neuroleptic-Naive First Episode Schizophrenic Patients.** *Neuropsychopharmacology (2008) 33, 2435-2441*

Terminology and Abbreviations

- **5-HT**: 5-hydroxytryptamine, serotonin
- **5-HT$_{2A}$**: The serotonin 2A receptor
- **AIC**: Akaike's Information Criterion
- **^{18}F-altanserin**: ^{18}F-labeled 3-(2-[4-(4-fluorobenzoyl)-1-piperidinyl]-ethyl)-2,3-dihydro-2-thioxo-4-quinazolinone.
- **BMI**: Body Mass Index = body weight/(height)^2 (kg/m^2)
- **BP_{ND}**: Binding potential, the ratio at equilibrium of specifically bound tracer to that of nondisplaceable tracer in tissue. (Outcome measure for ^{11}C-DASB PET)
- **BP_P**: Binding potential, the ratio at equilibrium of specifically bound tracer to that of total parent tracer in plasma. (Outcome measure for ^{18}F-altanserin PET)
- **^{11}C-DASB**: ^{11}C-labeled 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile
- **DSM-IV**: Diagnostic and Statistical Manual of Mental Disorders (version 4)
- **ICD-10**: International Statistical Classification of Diseases (version 10)
- **MDI**: Major (ICD-10) Depression Inventory
- **MDMA**: 3,4-methylenedioxy-N-methylamphetamine = Ecstasy
- **MRI**: Magnetic Resonance Imaging
- **NEOPI-R**: NEO Personality Inventory – Revised
- **PANNS**: Positive and Negative Syndrome Scale
- **PET**: Positron Emission Tomography
- **SCAN-2.1**: Schedules for Clinical Assessment in Neuropsychiatry (version 2.1)
- **SCL-90-R**: Symptom Check List Revised
- **SERT**: Serotonin transporter = 5-HTT
- **SPECT**: Single Photon Computed Tomography
- **SSRI**: Selective Serotonin Reuptake Inhibitor
- **VOI**: Volume of Interest

Background

The brain 5-HT system and its markers

Central serotonin (5-hydroxytryptamine, 5-HT) function has a role in normal brain function; it includes modulation of mood states, sex, hunger, sleep, memory, emotion, and endocrine responses. In addition, disturbances in the distribution and/or gene regulation of pre- and postsynaptic markers are believed to be implicated in the pathophysiology of conditions such as schizophrenia, eating disorders, mood disorders, as well as nicotine and alcohol dependence.

Two important markers within the 5-HT system, the serotonin transporter (SERT) and serotonin 2A receptor (5-HT_{2A}), will be in focus in this thesis. Their measurements with *in vivo* imaging will serve to address the suspected involvement of the serotonergic transmission, and in particular of these two markers, in the physiology and pathophysiology of eating behaviour, tobacco smoking, alcohol consumption, and schizophrenia, respectively. Here, in the Background section, an introduction is given to the 5-HT system in general and the SERT and 5-HT_{2A} receptors in particular. The background for investigating these markers in eating behaviour, tobacco smoking, alcohol consumption and schizophrenia is then presented in separate sections.

5-HT is synthesized and released by neurons that have their cell bodies in the raphe nuclei in the midbrain and through projections to every part of the brain (see **fig 1**). The signalling of the released 5-HT is mediated through at least 14 pre- and postsynaptic receptor proteins encoded by distinct genes (Gray and Roth 2001). The receptors can be divided into seven major classes: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 (Roth 2006). Most classes have several subtypes, including the 5-HT_2 class that can be divided into 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} (Kroeze and Roth 1998; Roth, Berry et al. 1998; Roth, Willins et al. 1998), and all of the 5-HT receptors, except for the 5-HT_3 subtype, are members of the G protein-coupled superfamily. Interest in the 5-HT_{2A} receptor subtype (Leysen, Niemegeers et al. 1978) has been prompted by increasing evidence of its involvement in a variety of neuropsychiatric disorders and in the therapeutic effect of the new generation of antipsychotics. In particular, this receptor is suspected to have a role in the pathophysiology behind important diseases like depression (Meyer, Kapur et al. 1999; Meyer, McMain et al. 2003; Mintun, Sheline et al. 2004; Bhagwagar, Hinz et al. 2006), schizophrenia (Dean 2003; Erritzoe, Rasmussen et al. 2008) and eating disorders (Kuikka,

Tammela et al. 2001; Kaye 2008). Moreover, the 5-HT_{2A} receptor appears to be the major site of action of hallucinogens (Nichols 2004). The receptor is heterogeneously distributed with very high receptor concentrations in several cortical areas, including frontal, parietal, temporal and occipital lobes (Pazos A 1987; Adams, Pinborg et al. 2004; Varnas, Halldin et al. 2004) where they are located post (and peri-) synaptically (Miner, Backstrom et al. 2003). Cerebellum has only negligible amounts of 5-HT_{2A} receptors (Pazos A 1987; Cortes, Soriano et al. 1988; Kish, Furukawa et al. 2005).

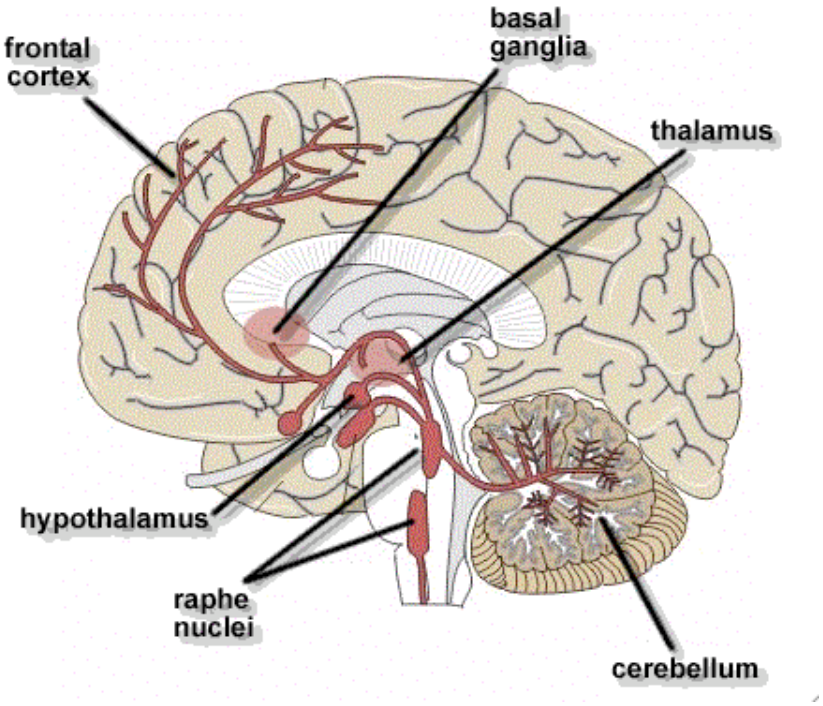


Figure 1

Projections from serotonergic neurons. *(Taken from Univ of Plymouth, Dpt of Psychology, Study and Learning Materials On-Line).*

Another interesting protein is the serotonin transporter (SERT) that is crucial for the regulation of 5-HT transmission as it controls the 5-HT availability at postsynaptic receptors by high affinity reuptake of released 5-HT (Blakely, De Felice et al. 1994). As shown in the diagram of a serotonergic chemical synapse (**fig 2**), SERT is located presynaptically on the serotonergic nerve terminal whereas e.g. 5-HT_{2A} receptors are situated postsynaptically.

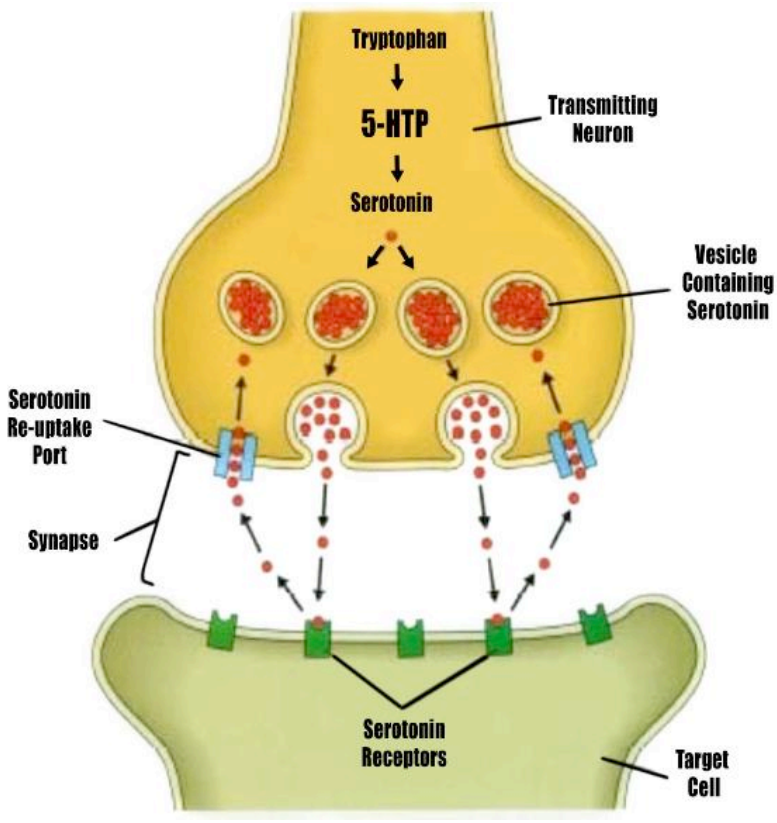


Figure 2

A serotonergic synapse. (*From: www.completehealthdallas.com)*

The SERT represents a molecular target for both antidepressants and drugs of abuse. By blocking or even reverting SERT, selective serotonin reuptake inhibitors (SSRIs) and illegal drugs such as MDMA (3,4-methylene-dioxy-methamphetamine, ecstasy) enhance synaptic 5-HT concentrations. Long term SERT blockade with SSRIs leads a number of different pre- and postsynaptic alterations (Hervas and Artigas 1998; Trillat, Malagie et al. 1998; Gardier, David et al. 2003; Zanoveli, Nogueira et al. 2007; Gunther, Liebscher et al. 2008), including an increase in extracellular 5-HT levels (Invernizzi, Bramante et al. 1994; Kreiss and Lucki 1995; Hajos-Korcsok, McTavish et al. 2000; Owen and Whitton 2005). Moreover, functional polymorphic variants of the promoter region of the SERT gene, 5-HTTLPR, have been identified and related to eating disorders (Matsushita, Suzuki et al. 2004; Steiger, Joober et al. 2005; Sookoian, Gemma et al. 2007;

Akkermann, Paaver et al. 2008; Fuemmeler, Agurs-Collins et al. 2008) as well as to anxiety-related traits such as neuroticism and vulnerability to depression (Caspi, Sugden et al. 2003; Hariri and Holmes 2006). Evidence from both *in vitro* and *in vivo* studies have suggested that the short (S) allele of this polymorphism confers decreased SERT expression and binding sites (Lesch, Bengel et al. 1996; Little, McLaughlin et al. 1998; Heinz, Jones et al. 2000), especially when taking triallelic variation in the 5-HTTLPR into account (Praschak-Rieder, Kennedy et al. 2007; Reimold, Smolka et al. 2007), although conflicting data exist (Greenberg, Tolliver et al. 1999; Mann, Huang et al. 2000; Preuss, Soyka et al. 2000; van Dyck, Malison et al. 2004; Parsey, Hastings et al. 2006). SERT is located in high density in midbrain and intermediate to high density in subcortical areas such as striatum and thalamus whereas there are relatively low concentrations in cortex (Varnas, Halldin et al. 2004). The cerebellum, except for the vermis part, has only negligible amounts of SERT (Kish, Furukawa et al. 2005).

The regulation of SERT and the 5-HT_{2A} receptors

Increased evidence suggests a link between variation in SERT levels, whether genetic in origin or not, and 5-HT_{2A} receptor function. Heterozygous (5-HTT+/-) and homozygous knock-out mice have changed 5-HT_{2A} receptor density when compared to their wildtype littermates. These mice have six-fold increase in extracellular 5-HT and a 60-80% reduction in intracellular 5-HT concentrations (Bengel, Murphy et al. 1998; Montanez, Owens et al. 2003; Mathews, Fedele et al. 2004). Antagonist radioligand 5-HT_{2A} receptor binding is globally decreased in these animals (Rioux, Fabre et al. 1999) whereas a regional variation with decreased binding in striatum but increased binding in hypothalamus is seen with an agonist ligand (Li, Wichems et al. 2003). The 5-HT_{2A} receptor mediated serotonergic signaling is also markedly reduced in SERT knock-out mice (Qu, Villacreses et al. 2005). Likewise, chronic blockade for SERT decreases 5-HT_2 receptor responsiveness in rats (Maj and Moryl 1993; Kennett, Lightowler et al. 1994; Yamauchi, Miyara et al. 2006). Accordingly, transgenic mice that over-express SERT show increased 5-HT_{2A} receptor function even though the mRNA expression and the binding levels are unchanged (Jennings, Sheward et al. 2008). Since 5-HT depleted wildtype mice also have increased 5-HT_{2A} receptor function, this effect is possibly due to the low 5-HT levels that ensue the high SERT expression in these animals (Heal, Philpot et al. 1985; Godfrey, McClue et al. 1988).

Both 5-HT_{2A} receptors and SERT leves are sensitive to manipulations of 5-HT

levels. Experimental data suggest that SERT binding and cerebral 5-HT levels have an inverted U-shaped relation, with decreased SERT binding levels both after 5-HT depletion (Rattray, Baldessari et al. 1996; Rothman, Jayanthi et al. 2003) and after SSRI treatment leading to augmentation of 5-HT (Pineyro, Blier et al. 1994; Benmansour, Cecchi et al. 1999; Horschitz, Hummerich et al. 2001; Benmansour, Owens et al. 2002; Gould, Pardon et al. 2003; Gould, Altamirano et al. 2006). For the 5-HT_{2A} receptors, a negative relation with 5-HT levels is observed, with an increase in 5-HT_{2A} receptor binding after partial depletion (Heal, Philpot et al. 1985; Cahir, Ardis et al. 2007) and decreased binding after chronically increasing 5-HT levels with SSRI treatment (Nelson, Thomas et al. 1989; Cowen 1990; Maj, Bijak et al. 1996; Gunther, Liebscher et al. 2008) (**fig 3**). Also in humans, SSRI treatment has been related to decreased cortical 5-HT_{2A} receptor binding (Spigset and Mjorndal 1997; Meyer, Kapur et al. 2001).

Taken together, the observations from the experimental studies presented above suggest that there is a relationship between SERT and 5-HT_{2A} receptor levels. Secondly, we will argue that if such a co-regulation is mediated through individual 5-HT changes, then the predicted relationship would not be linear but rather an inverted U-shape.

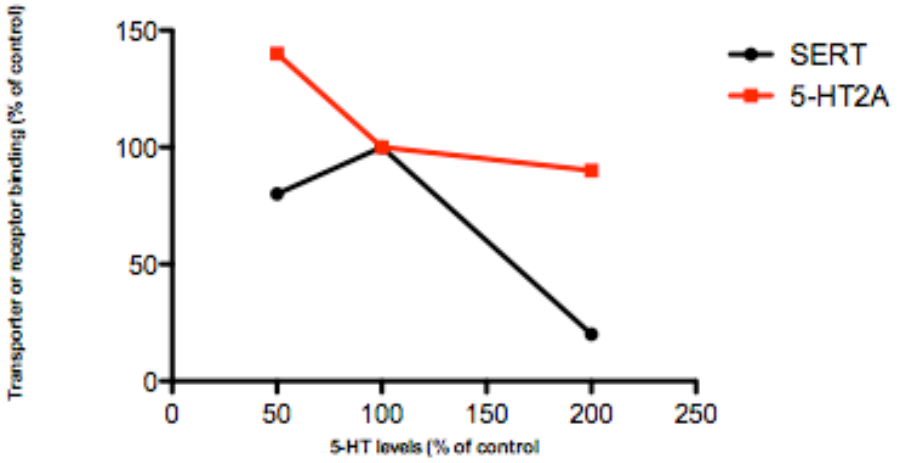


Figure 3

"*Model of in vitro brain 5-HT_{2A} receptor and SERT binding as a function of central 5-HT levels*". The model is based on in vitro autoradiography studies of brain 5-HT_{2A} receptor and 5-HTT binding in response to chronic 5-HT depletion and SSRI administration in rats. Central 5-HT tissue levels were reported in each 5-HT depletion study. However, as 5-HT levels were not reported in the SSRI administration studies, separate reports of extracellular brain 5-HT levels after chronic SSRI administration in rats were used. The model is a compilation of several studies and indicates directionality and proportionality of change but should not be perceived as quantitative or exact.

Obesity and the role of serotonergic neurotransmission in regulation of body weight

Obesity, a major nutritional disorder defined as abnormal or excessive accumulation of fat that may impair health, poses a major and increasing health threat in both the industrialized and industrializing world (**fig 4**). Obesity and overweight are important risk factors for developing a variety of disorders, including type-2 diabetes, cardio-vascular diseases, and certain types of cancer. Body mass index (BMI) is used as a convenient measure for the nutrition state of an individual and is known to generally correlate well with other anthropometric measures such as waist circumference (Molarius and Seidell 1998). A BMI of more than 25 kg/m^2 is defined as overweight, and a BMI over 30 kg/m^2 as obese (BMI= the weight in kilograms divided by the square of the height in meters (kg/m^2)). According to the latest data from World Health Organization (WHO 2006), approximately 1.6 billion adults globally are overweight, and at least 400 million of them are obese. It is estimated that diseases related to overweight cost up to 7% of the total health-care budget in several developed countries (WHO 2006). From experimental models of obesity as well as from twin and adoption studies, it is known that the development of obesity results from an interaction of both environmental and genetic factors (Eikelis and Esler 2005).

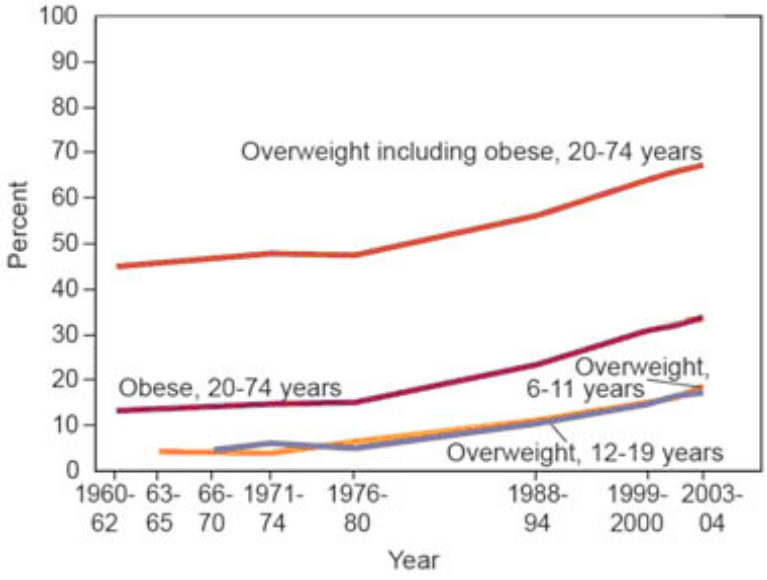


Figure 4

The prevalence of overweight and obesity in the United States.

(From Weight-control Information Network, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)).

About 30 years ago, the first studies suggested that 5-HT was important for control of food intake and appetite (Blundell 1977; Coscina and Stancer 1977; Hoebel, Zemlan et al. 1978; Blundell and Latham 1979). It was discovered that administration of agents that are either toxic to 5-HT neurons (e.g. 5,7-dihydroxytryptamine, 5,7-DHT) or prevent 5-HT synthesis (e.g. parachlorophenylanine, pCPA) increase food intake in rats with a

subsequent increase in body weight (Saller and Stricker 1976; Waldbillig, Bartness et al. 1981). In contrast, increased central 5-HT levels following administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP) or of the 5-HT releasing agent fenfluramine significantly decreased food intake (Clineschmidt 1973; Barrett and McSharry 1975; Blundell and Leshem 1975; Duhault, Boulanger et al. 1975). In accordance with these early results, both experimental and clinical administration of the 5-HT releasing agent fenfluramine, the selective serotonin reuptake inhibitor (SSRI) fluoxetine, the serotonin and norepinephrine reuptake inhibitor (SNRI) sibutramine, the 5-$HT_{1B/2C}$ agonist mCPP, and the 5-$HT_{1B/1D}$ agonist sumatriptan all lead to reduced food intake and subsequent weight loss (Halford, Harrold et al. 2005). Serotonergic drugs can both accelerate the onset of satiety (Blundell 1986; Li, Spector et al. 1994), enhance basal metabolic rate, and inhibit carbohydrate craving (Moses and Wurtman 1984; Laferrere and Wurtman 1989).

Both genetic and pharmacological studies have identified the relevant 5-HT receptor subtypes that are implicated in regulation of energy balance. Selective activation of 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors leads to hypophagia in various animal models (Dourish 1995; Bickerdike, Vickers et al. 1999). Although most focus in this line of research has been on the 5-HT_{2C} receptor, a specific role of the 5-HT_{2A} receptor in the regulation of body weight has also been suggested in different types of studies. E.g., G/G carriers of the A(-1438)G promoter polymorphism of the 5-HT_{2A} receptor gene have increased body mass and predominantly abdominal distribution of body fat (Rosmond, Bouchard et al. 2002). Some studies argue that A carriers of the same polymorphism are at higher risk of developing anorexia nervosa but the results are mixed (Ricca, Nacmias et al. 2002). In further support of a relationship between 5-HT_{2A} receptor binding and regulation of eating, PET and SPECT studies have suggested that both anorexia nervosa patients and patients recovered from regular anorexia nervosa and bulimia-type anorexia nervosa display decreased cerebral 5-HT_{2A} receptor binding (Kaye, Frank et al. 2005). Furthermore, mice that became obese by exposure to high-fat diet showed increased 5-$HT_{2A/2C}$ receptor density in comparison to obese-resistant mice fed on the same diet (Huang, Huang et al. 2004). Finally, in a sample of 52 healthy, largely normal-weighted subjects, we have previously identified a positive correlation between BMI and 5-HT_{2A} receptor binding in the left superior temporal cortex, left medial inferior temporal cortex, right dorsal lateral prefrontal cortex, and right sensory motor cortex ($p<0.0125$)(Adams, Pinborg et al. 2004).

In addition to the suggested implication of postsynaptic serotonergic markers in the

pathophysiology of overweight/obesity, SERT regulation also seems to play a role. It has been shown that SERT mutant (SCL6A4-/-) mice become obese (Murphy and Lesch 2008), and in obese and overweight individuals recent evidence points to a decreased expression of the gene encoding for SERT (Sookoian, Gemma et al. 2007; Fuemmeler, Agurs-Collins et al. 2008). Only few in vivo molecular imaging SERT studies have been performed on overweight/obese subjects. In a SPECT study, midbrain SERT binding was found to be lower in binge eating obese women than in non-binging obese women (Kuikka, Tammela et al. 2001) and at the re-examination after 8-24 months of SSRI treatment SERT binding in the binge eating obese subjects was increased (Tammela, Rissanen et al. 2003). In a more recent [^{123}I]nor-β-CIT SPECT study on 16 monozygotic twin pairs, twins with a BMI higher than their monozygotic co-twins were found to have higher SERT binding (Koskela, Kaurijoki et al. 2008).

The role of serotonergic neurotransmission in tobacco smoking and alcohol consumption.

Serotonergic neurotransmission has also been related to both alcohol consumption and tobacco smoking. Administration of 5-HT enhancing drugs reduces voluntary alcohol consumption in both animals and humans (Lyness and Smith 1992; Naranjo, Poulos et al. 1992; Sellers, Higgins et al. 1992; McBride, Bodart et al. 1995) and conversely, 5-HT depletion leads to increased alcohol craving (Lyness and Smith 1992). Further, deficiency of brain 5-HT and its metabolites has been detected in rats genetically selected for their preference to alcohol (Murphy, McBride et al. 1983; Zhou, Bledsoe et al. 1991; McBride, Bodart et al. 1995). An association between alcohol dependence and decreased SERT expression/density in humans has been suggested (Heinz, Goldman et al. 2004; Feinn, Nellissery et al. 2005). Parallel observations have been made for the relation between tobacco smoking and 5-HT transmission. Thus, enhancement of serotonergic neurotransmission decreases nicotine intake (Opitz and Weischer 1988), whereas repeated nicotine treatment decreases frontocortical 5-HT levels in animals (Harrison, Everitt et al. 1997; Olausson, Engel et al. 2002). Used in combination with transdermal nicotine substitution therapy, SSRI has furthermore been shown to increase abstinence rates over placebo (Killen, Fortmann et al. 2000) even though a central role of SSRI treatment for smoking cessation has recently been questioned in Cochrane review (Hughes, Stead et al. 2007).

Schizophrenia

In the research pointing to a role of serotonergic neurotransmission in physiology behind eating behaviour, animal models have played an important role, as indicated earlier in this section. Given the complexity of a disease like schizophrenia, the use of animal models for such a condition is less evident, and the background for suspecting disturbances in serotonergic transmission, and in particular in the regulation of the 5-HT_{2A} receptor, therefore primarily has come from observations in humans, although animal studies also have provided significant knowledge to this field of research.

Schizophrenia is a severe chronic neuropsychiatric disorder, associated with high prevalence (approximately 1% in the general population). Symptoms of schizophrenia typically emerge during early adulthood and are usually classified as positive, negative, or cognitive symptoms. Positive symptoms include delusions, hallucinations (typically auditory), and severe thought disorganization. Negative symptoms comprise apathy, poverty of speech, anhedonia, and social withdrawal. Cognitive symptoms, such as deficits in attention and memory, are also prominent features of the illness. The etiology and the pathophysiology of schizophrenia remain unclear, but dysregulations in several neurotransmitter systems, including the dopamine, GABA, glutamatate, cholinergic, and serotonin system seems to be a prominent feature. Among these, the dopamine system has received most attention. The classic dopamine hypothesis, formulated more than 30 years ago, propose that the disease is due to hyperactivity of the dopaminergic transmission (Carlsson and Lindqvist 1963; Rossum 1966). With a later modification of this hypothesis, the current predominant view is that dopamine systems in schizophrenia might be characterized by an imbalance between subcortical and cortical dopamine neurotransmission, with hyperactive mesolimbic and hypoactive mesocortical dopamine projections (Weinberger 1987; Knable and Weinberger 1997; Goldman-Rakic, Muly et al. 2000).

5-HT_{2A} receptors in schizophrenia

In addition to a role of the dopamine system, the observation that lysergic acid diethylamide (LSD) - a drug with structural similarities to 5-HT and high affinity to 5-HT_{2A} receptors - has hallucinogenic properties similar to schizophrenic symptoms led to the hypothesis that 5-HT may be involved in the pathogenesis of schizophrenia (Gaddum 1954; Wooley and Shaw 1954; Aghajanian and Marek 2000). Subsequently, dysfunction in the 5-HT system in schizophrenia has been supported by evidence from cerebrospinal fluid and

postmortem studies, as well as by studies using pharmacological challenges (Abi-Dargham, Laruelle et al. 1997). Further, indirect support for the involvement of the 5-HT_{2A} receptor in this disorder comes from the association between the receptor profile and clinical characteristics of newer atypical antipsychotic drugs. Compared to typical antipsychotic drugs, which primarily bind to the dopamine D_2 receptors, most atypical antipsychotic drugs have higher affinity to cortical 5-HT_{2A} receptors than to striatal D_2 receptors (Roth, Hanizavareh et al. 2004). This feature might explain why 5-HT_{2A} receptor antagonist treatment has a relatively good effect on the negative and cognitive symptoms and at the same time is associated with minimal extrapyramidal side effects (EPS) (Tyson, Roberts et al. 2004). It has, on the other hand, also been questioned whether the 5-HT_{2A} receptor has such a central role in the antipsychotic effect of atypical antipsychotics (Kapur and Seeman 2001). There are at least two antipsychotic drugs with atypical properties, amisulpride and remoxipride, that both lack any relevant affinity for the 5-HT_{2A} receptor.

As reviewed in **table 1**, eleven out of fifteen post-mortem brain studies have reported a decreased 5-$HT_{2A/C}$ receptor binding schizophrenic patients, in cortical areas, and mostly in frontal cortex. So far, only four in vivo imaging studies using PET in first episode antipsychotic-naïve schizophrenic patients have been carried out, and the results are inconsistent. Three studies found no difference in 5-HT_{2A} receptor binding between schizophrenic patients and healthy controls (Trichard, Paillere-Martinot et al. 1998; Lewis, Kapur et al. 1999; Okubo, Suhara et al. 2000) and one study found a decreased binding in the left lateral frontal cortex in the patient group (Ngan, Yatham et al. 2000). However, these studies are limited by small sample sizes and usage of the radioligands such as ^{18}F-setoperone and ^{11}C-N-methylspiperone which have a relatively poor 5-HT_{2A} receptor selectivity.

Table 1: The 5-HT2A receptor in human brain: Post-mortem and *in vivo*.

Type of study	Reference	Method	Radioligand	Number of patients/ctr	Outcome in schizophrenic patients when compared to controls
Postmortem	(Bennet 1979)	Membrane	[^{3}H]LSD	21/31	⇓ density in frontal cortex
	(Whitaker, Crow et al. 1981)	Membrane	[^{3}H]LSD	13/8	B_{max}⇒ , K_d ⇑
	(Reynolds, Rossor et al. 1983)	Membrane	[^{3}H]ketanserin	11/10	⇒ in cortical density
	(Mita, Hanada et al. 1986)	Membrane	[^{3}H]ketanserin	11/16	B_{max}⇓ in cortex, K_d ⇒
	(Arora and Meltzer 1991)	Autoradiography	[^{3}H]ketanserin	20/20	Cortical B_{max}⇓, K_d ⇒
	(Joyce, Shane et al. 1993)	Autoradiography	[^{3}H]LSD	10/8	B_{max} ⇑ in ventral putamen, nucl. acc., post. cingulate, hippocampus, ⇒ in frontal cortex, K_d ⇒
	(Laruelle, Abi-Dargham et al. 1993)	Membrane	[^{3}H]ketanserin	6/13	Cortical B_{max} ⇓ in non-suicide psychotics, K_d ⇒
	(Dean, Hayes et al. 1996)	Membrane	[^{3}H]ketanserin	20/20	B_{max}⇒, K_d ⇒
	(Burnet, Eastwood et al. 1996)	Autoradiography	[^{3}H]ketanserin	13/15	Cortical B_{max}⇓
	(Dean and Hayes 1996)	Autoradiography	[^{3}H]ketanserin	20/20	Frontal B_{max}⇓
	(Gurevich and Joyce 1997)	Autoradiography	[^{3}H]LSD	10/12	Frontal and parietal B_{max}⇓ in patients on antipsychotics at death. BA 6 and 24 ⇓ prefrontal cortex B_{max} in patients off antipsychotics at death
	(Dean, Hayes et al. 1998)	Autoradiography	[^{3}H]ketanserin	55/55	Frontal B_{max}⇓
	(Dean, Hussain et al. 1999)	Autoradiography	[^{3}H]ketanserin	19/19	Frontal B_{max}⇓
	(Pralong, Tomaskovic-Crook et al. 2000)	Membrane Autoradiography	[^{3}H]ketanserin	10/10 20/20	Planum temporale B_{max}⇓ K_d ⇓ in subjects treated with phenothiazibes
	(Matsumoto , Inoue et al. 2005)	Autoradiography	[^{3}H]ketanserin	6/6	Prefrontal density ⇓ ⇒ in strital density
PET	(Trichard, Paillere-Martinot et al. 1998)	PET	[^{18}F]setoperone	14(7*)/15	Cortical binding ⇒, no subcortical data reported.
	(Lewis, Kapur et al. 1997)	PET	[^{18}F]setoperone	13(10*)/26	Cortical binding ⇒, no subcortical data reported.
	(Okubo, Suhara et al. 2000)	PET	[^{11}C]N-methylspiperone	17(10*)/12	Cortical binding ⇒ , no subcortical data reported.
	(Ngan, Yatham et al. 2000)	PET	[^{18}F]setoperone	6(6*)/6	Frontal cortex ⇓

*) Number of neuroleptic-naïve patients. Receptor binding: ⇓ = Decrease, ⇑ = Increase, ⇒ = No change.

Aims and hypothesis

Aim 1:

To test in healthy human subjects whether BMI, alcohol consumption, and tobacco smoking were associated with changes in the cerebral 5-HT_{2A} receptor binding using ^{18}F-altanserin-PET. A positive relation between 5-HT_{2A} receptor binding and BMI, degree of alcohol consumption and tobacco smoking was hypothesized.

Aim 2:

To test in healthy human subjects whether BMI was associated with changes in the *in vivo* cerebral SERT binding using ^{11}C-DASB-PET. A negative correlation was expected. The relationship between SERT binding and alcohol consumption and tobacco smoking was also tested.

Aim 3:

To investigate cortical 5-HT_{2A} receptor binding in a group of first episode antipsychotic-naïve schizophrenic patients and matched healthy controls using ^{18}F-altanserin-PET. It was the expectation to find a decreased cortical 5-HT_{2A} receptor binding in schizophrenic patients.

Aim 4:

To compare SERT and 5-HT_{2A} receptor binding levels in the same subjects to establish if these two markers of the serotonergic system covariate. An inverted U-shaped relation was hypothesized.

Methods

Subjects

The subjects were recruited by newspaper and internet advertisement (study 1 and 2 and controls from study 3) and after voluntary first-time referral to a psychiatric unit (schizophrenic patients in study 3). They all received a full explanation of the study and providing written informed consent according to the declaration of Helsinki II before their participation. Study protocols were approved by the Ethics Committee of Copenhagen and Frederiksberg. In total, 159 subjects (144 healthy and 15 schizophrenic patients) participated in the studies that encompass this PhD-thesis. Many of the healthy subjects participated in more than one study, for overview please see **fig 5.**

For all subjects (except for the schizophrenic patients in study 3) the following inclusion criteria were fulfilled:

- lifetime naïve to antidepressants and antipsychotics
- no stimulant abuse
- no history of neurological or psychiatric disorders
- Symptom Check List Revised (SCL-90-R) scores below cut-offs for psychopathology.
- normal neurological examination, no contraindications to MR imaging, no significant head injury
-

Aim 1 (Study 1):

<u>136 adult subjects</u>

- 51 females, 84 males
- Mean age: 40.5 ± 18.6 years
- Mean BMI: 25.2 ± 4.3 kg/m^2 , 14 out of 136 were obese (BMI above 30).

Aim 2 (Study 2):

<u>60 adult subjects</u>

- 23 females, 37 males
- mean age: 35.7 ± 18.2 years
- mean BMI: 26.5 ± 5.9 kg/m^2, 7 out of 60 were obese (BMI above 30)

Aim 3 (Study 3):

15 schizophrenic patients

- 4 females, 11 males
- mean age: 27.5 ± 4.5 years
- mean BMI: 23.7 ± 2.5 kg/m^2
- fulfilled ICD-10 and DSM IV criteria for schizophrenia (diagnosed using SCAN 2.1)
- antipsychotic-naïve
- normal neurological examination, no contra indications to MR imaging, no significant head injury
- Except for one, none of the patients used any drugs of abuse or fulfilled ICD-10 or DSM IV criteria for either drug abuse or drug dependence
- 4 patients had prior (n=3) or present (n=1) use of antidepressant medication (SSRI).

15 healthy controls

- matched for age, gender and BMI.
- 4 females, 11 males
- mean age: 28.5 ± 5.7 years
- mean BMI: 23.4 ± 2.2 kg/m^2

Aim 4 (Additional analysis of relationship between in vivo SERT and 5-HT_{2A} receptor binding levels in the same subjects):

56 adult subjects

- 22 females, 36 males
- mean age: 36 ± 19 years
- mean BMI: 26.5 ± 6.0 kg/m^2

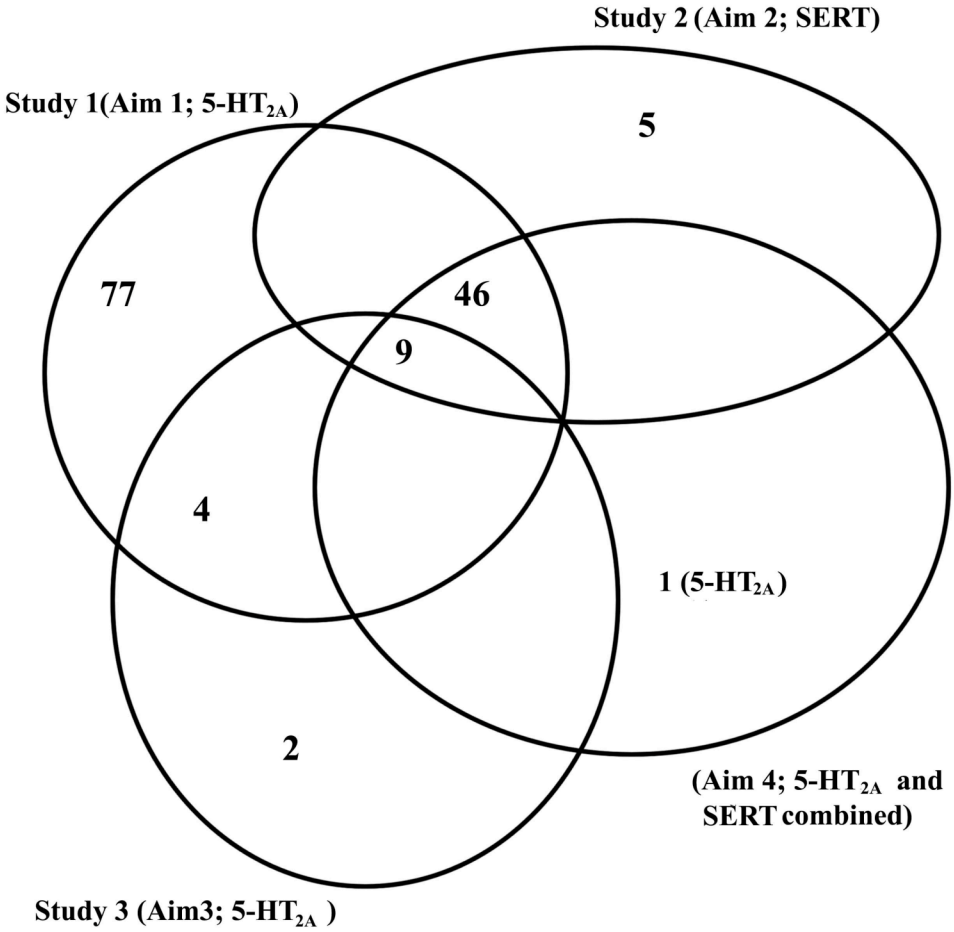


Figure 5

The distribution of the 144 healthy controls that went into this PhD-thesis.

Imaging

Measuring serotonergic neurotransmission in vivo in humans with PET

With Positron Emission Tomography (PET) it is possible to measure receptor/transporter binding in the living human brain given that a suitable radiolabeled tracer is available for the marker at interest. General tracer requirements include: 1) the ligand should readily be labeled with a PET isotope with an appropriate half-life (either ^{11}C or ^{18}F), 2) the radiolabeled tracer should pass the blood brain barrier in sufficient amounts, 3) the radioligand should preferably not yield any radiolabeled metabolites crossing the blood brain barrier, 4) a favourable ratio between tracer uptake in specific versus non-specific binding tissue, 5) fast kinetics allowing quantification by kinetic modeling or fast achievement of steady state between tracer in plasma and in brain tissue, 6) tolerable radioactivity dosages (ideally below 5 mSv), and 7) tolerable scanning paradigm (short acquisition time e.g. <90 min). Further, a suitable reference region for the tracer should also be present in order to provide a measure for the non-specific binding. The use of a reference region sometimes enables quantification without the need for arterial input measurements which is an advantage (Lammertsma and Hume 1996).

The following section briefly describes and discusses the methods we applied to image and quantify the 5-HT_{2A} receptor and SERT binding. The methods are also described in more detail in the papers.

5-HT_{2A} receptor imaging with ^{18}F-altanserin PET

Currently, two selective radioligands are employed for PET-imaging 5-HT_{2A} receptors: ^{18}F-altanserin and ^{11}C-MDL100907. In addition, ^{18}F-setoperone has been used but its moderate affinity for the dopamine D_2 receptor only allows interpretation of 5-HT_{2A} receptor binding in brain regions with low D_2 receptor density, i.e., mainly in cortex. The ^{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 5-HT_{2A} receptor antagonist. ^{18}F-altanserin binds with high selectivity and high affinity to the 5-HT_{2A} receptor (Kristiansen, Elfving et al. 2005). The cerebellum contains negligible levels of 5-HT_{2A} receptors (Pazos, Probst et al. 1987) and can be employed as a reference region. Metabolite measurements, acquisition procedures, reconstruction, and quantification are described in the method section of paper 1 and 3 and by (Pinborg, Adams

et al. 2003). The outcome parameter is defined as follows:

$$BP_P = (C_{VOI} - C_{Reference}) / C_{Plasma} = f_p * (B_{max}/K_d)\ (mL/mL) \qquad \textit{Equation 1}$$

where C_{VOI} 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 has in our lab been determined to be 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 5-HT_{2A} receptor binding (Haugbol, Pinborg et al. 2007).

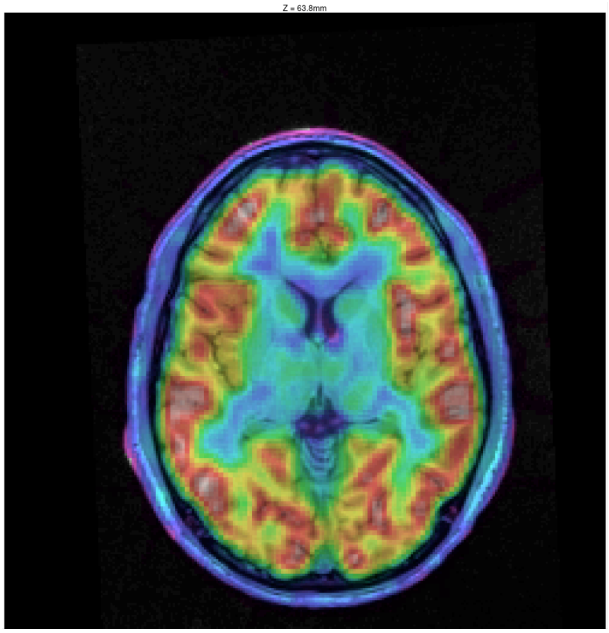


Figure 6

Transversal ^{18}F-altanserin PET aligned to the same individual's MR image, from a 23 y.o. male. Note the high cortical tracer uptake.

SERT imaging with ^{11}C-DASB PET

A wide range of candidate radiotracers developed to allow the measurement of SERT binding have not proven suitable for *in vivo* imaging (Smith 1999; Wilson, Ginovart et al. 2000; Huang, Bae et al. 2001; Huang, Hwang et al. 2002). The most frequent shortcomings have been: Lack of selectivity over especially the dopamine transporter (DAT), poor signal to noise ratios, and inappropriate pharmacokinetics, often with slow clearance of non-specific binding. However, since its discovery and validation in the beginning of this millennium, ^{11}C-labeled 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile (^{11}C-DASB) has become one of the favorite PET radiotracers due to its high specificity, fast kinetics, and favourable signal to noise ratio (Houle, Ginovart et al. 2000; Ginovart, Wilson et al. 2001; Huang, Hwang et al. 2002; Frankle,

Slifstein et al. 2006; Kim, Ichise et al. 2006). In study 2, SERT binding is imaged with ^{11}C-DASB PET as illustrated in **fig 7**. Our measurements are based on 90 minutes dynamic acquisition starting immediately after bolus injection, according to Ichise et al (Ichise, Liow 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, Ichise et al. 2006).

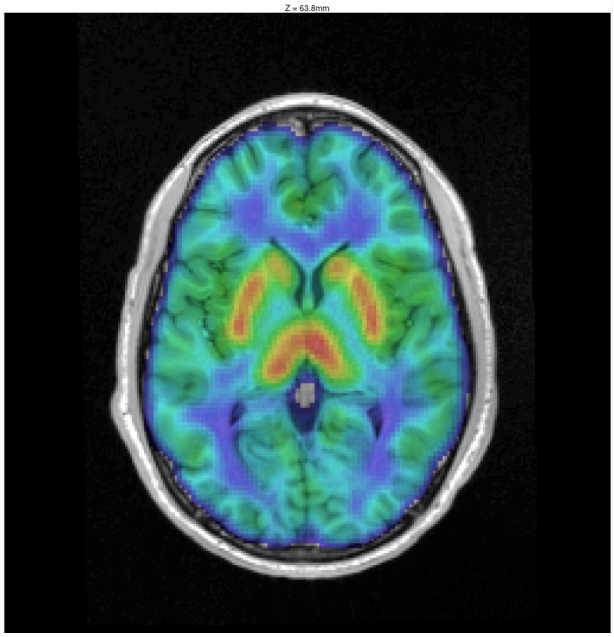


Figure 7

Transversal [^{11}C]DASB-PET aligned to the same individual's MR image, from a 23 y.o. male. Note the high tracer uptake in striatum and thalamus.

Structural brain imaging with magnetic resonance

As a supplement to all PET-studies, magnetic resonance imaging (MRI) is conducted 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 study 2, 3, and for aim 4, all subjects were MR-scanned using a 3 Tesla Trio scanner whereas about half of the subjects (n=67) in study 1, were scanned on a 1.5 Tesla Vision scanner. This was because the MR Department at Hvidovre Hospital

changed their scanner during the time period where subjects were included for PET imaging with ^{18}F-altanserin.

Volumes of interest were delineated in a strictly user-independent fashion as earlier described (Svarer, Madsen et al. 2005). With this approach, a template set of 10 MRIs is automatically co-registered to a new subject's MRI. The identified transformation parameters are used to define volumes of interest (VOIs) in the new subject MRI space and through the co-registering these VOIs are transferred onto the PET images. Main VOIs in study 1 and 3 were the cortical regions where 5-HT_{2A} receptor density is high, whereas the main VOIs in study 2 were the subcortical high-binding SERT region, caudatus-putamen-thalamus, as well as the midbrain. Details about MR acquisition, bias correction, and segmentation of the MRIs are described in the papers.

Genotyping in study 1 and 2

Blood was drawn on the day of the ^{18}F-altanserin or ^{11}C-DASB PET scan and genomic DNA was purified from the buffy coat by standard methods. In study 1, ninety-five subjects were genotyped for determination of the 5-HT_{2A} receptor gene G(-1438)A (rs6311) promoter polymorphism. Genomic DNA was extracted from whole blood, specifically buffy coat lymphocytes, using a purification set from Qiagen Incorporate (www.qiagen.com). The 5-HT_{2A} G(-1438)A promoter polymorphism was identified by using the PCR protocol described by Masellis et al. (Masellis, Basile et al. 1998). In short, PCR amplification of a 200 base pairs (bp) fragment was generated by forward primer 5'-CTA GCC ACC CTG AGC CTA TG-3' and reverse primer 5'-TTG TGC AGA TTC CCA TTA AGG-3' and followed by restriction enzyme digestion with MspI for 4 h. PCR fragment were separated on a 2% agarose gel (SeaKem GTG Agarose, www.cambrex.com). An A at position -1438 leads to an uncut fragment of 200 bp and a G at position -1438 leads to two fragments of length 121 bp and 79 bp.

Fourty-nine out of the 60 subjects scanned with ^{11}C-DASB were genotyped for the 5-HTT-linked polymorphic region 5-HTTLPR. The 5-HTTLPR long (A/G) polymorphism was detected by MspI restriction enzyme digestion of the PCR products and the generated fragments were resolved by gel electrophoresis. Product sizes for the digest were in line with the literature: L_A=340 bp, L_G=166 bp+174 bp, S=297 bp (Praschak-Rieder, Kennedy et al. 2007).

BMI, personality, and psychiatric symptoms

On the day of the PET scan, all subjects were weighted and measured. Body Mass Index was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). In 36 persons, waist circumference was also measured. All healthy subjects also completed the Symptom Check List Revised (SCL-90-R) in order to assess symptoms of distress and psychopathology (Derogatis 1994), and the Major (ICD-10) Depression Inventory (MDI) questionnaire to further assess depressive symptoms (Bech, Rasmussen et al. 2001). The Danish version of the 240-item NEOPI-R self-report personality questionnaire (Hansen 2004) was also filled in by the subjects on the day of the PET scan. The NEO-PI-R evaluates the broad personality dimensions of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness.

Statistical approach in the three studies

The association between the binding of the two markers and BMI is modeled using normal linear regression with adjustment for appropriate covariates (study 1 and 2). In addition, Students unpaired t-test is used when comparing different variables in normal weigted vs overweight/obese subjects. A t-test was also employed when comparing 5-HT_{2A} receptor binding and other parameters in schizophrenic patients vs matched controls (study 3). Detailed information about the employed models is included in the individual method sections of the three studies.

Here, the background for the variables included for adjustment purposes in the analysis is presented:

In healthy volunteers the 5-HT_{2A} receptor (Adams, Pinborg et al. 2004) and (for some studies, at least) SERT binding (Pirker, Asenbaum et al. 2000; van Dyck, Malison et al. 2000; Meyer, Wilson et al. 2001; Reimold, Smolka et al. 2007) show an age dependent decrease, and, as reported, age adjustment was also appropriate in our studies. In study 1, the 5-HT_{2A} receptor binding was also corrected for neuroticism score and type of MR scanner. The first variable was included because an association between the personality trait neuroticism and 5-HT_{2A} receptor binding has been demonstrated (Frokjaer, Mortensen et al. 2008), the latter because two types of MR scanners (1.5 and 3.0 T) were employed (for considerations with regard to MR imaging, please see below).

The SERT binding assessed in study 2 was in addition to age also adjusted for gender, minutes of daylight on the scan date, and openness to experience. Gender was included in the linear regression model because of a suggested influence of sex on SERT

binding (Staley, Krishnan-Sarin et al. 2001; Jovanovic, Lundberg et al. 2008). The other two variables were included because of observations of relations with SERT binding (Kalbitzer, submitted).

Statistical approach for aim 4

Since the comparative analysis of the binding levels of two markers within 56 subjects is not included in any of the three papers, a more detailed description of this analysis is provided.

To explore the association between 5-HT_{2A} receptor binding (BP_p) in neocortex and SERT binding (BP_{nd}) in midbrain, the subcortical high-binding region, and neocortex, respectively, a non-parametric model using penalized regression splines with the smoothness automatically selected by a Generalized Cross Validation (GCV) criterion Wood (2008 REF) was first applied. Second, the aim was a parametric model allowing for an easier interpretation. Based on the non-parametric regression analysis, the association between the two markers was subsequently modeled using polynomials of the 5-HT_{2A} BP_p up to order three. To assist the model selection, Akaike's Information Criterion (AIC) was employed.

Methodological considerations

In this section, concerns related to the different methods employed in this thesis will be addressed.

PET: Both ligands

Different amounts of injected unlabeled ligand could lead to differences in binding potentials; injection of a too high mass of the unlabeled compound results in an underestimation of the binding potential because of competitive binding between labeled and unlabeled ligand. Therefore, the amount of given mass was checked in the 3 studies, although only indirectly in study 3 (specific activity and injected hot dose evaluated separately). No between-group differences were detected in any of the 3 studies.

For aim 4, half of the 56 subjects (n=28) were investigated with both radioligands on the same day and the remaining subjects were investigated with number of days between the two scans ranging from 0 to 672. The markers might change over time and thus not

reflect covariation if they are not measured close to each other in time. Therefore, in a post-hoc analysis, the quadratic effect was estimated in a subsample of individuals with maximum 2 weeks interval between the two measurements. The relationship between the two markers did not differ from what was detected in the total sample as illustrated in **fig 8**.

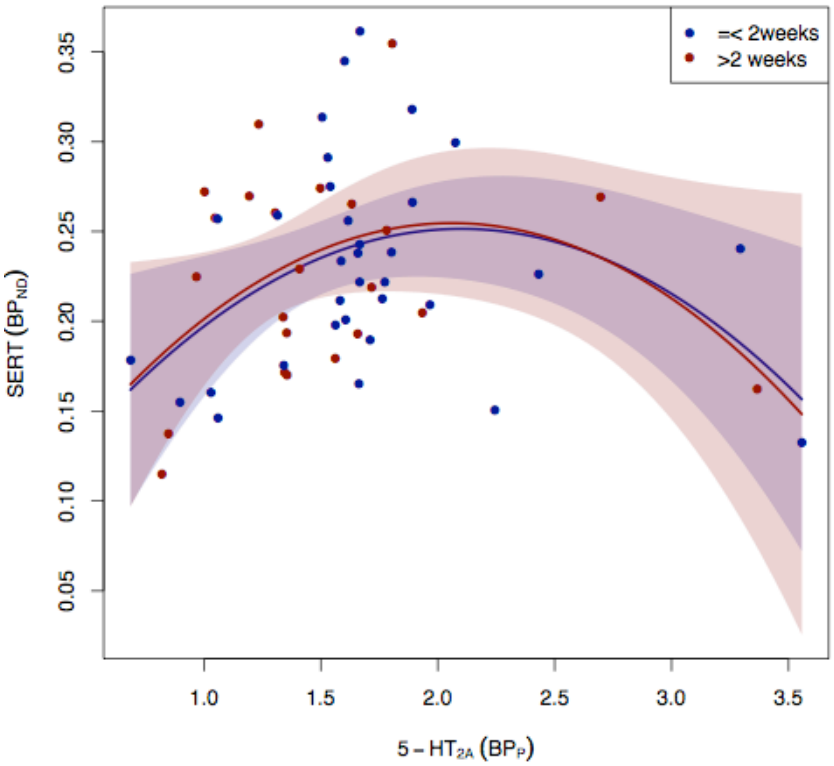


Figure 8

The estimated quadratic functional form of cortical 5-HT_{2A} vs cortical SERT binding for individuals with maximum 2 weeks and minimum 2 weeks interval between the two measurements, respectively. Null-hypothesis no difference: p=0.99, 3 degrees of freedom.

Comorbidity between affective disorders and obesity exist: Patients suffering from major depressive disorder have higher frequency of overweight, obesity, and abdominal obesity; and obese persons seeking weight-loss treatment have higher frequency of depressive and bipolar disorders (McElroy, Kotwal et al. 2004). For this reason, and because of the known or suspected involvement of both SERT and 5-HT_{2A} receptors in the pathophysiology of a variety of neuropsychiatric disorders, it could be speculated if the detected relationships between BMI and binding potentials of the two markers could be caused by differences in psychiatric symptoms such as depression- or anxiety- like symptoms in overweight/obese as compared to normal weighted subjects. To evaluate if

this was the case, scores from SCL-90-R as well as MDI was compared between normal weighted and overweight/obese subjects in study 1 and 2. No significant between group differences were detected for any of these measures incl submeasures of SCL-90-R (a part of these data are presented in paper 1 and 2, the rest is not shown) indicating that the found relationships with BMI is not due to differences in psychiatric symptoms.

The outcome measure for both markers, the binding potential (BP), reflects the product of the number of binding sites (B_{max}) and the affinity ($1/K_d$). Thus, the measured inter-individual variation in BPs for both markers could be attributed to changes in both of these parameters. In vitro, it is possible to derive K_d and B_{max} by altering the concentration of the unlabeled ligand. In vivo studies of this type cannot easily be performed in humans given the high concentration of unlabeled drug required. In one human PET study, however, a saturation analysis revealed a significant interindividual variability in B_{max} but not in K_d (Farde, Hall et al. 1995). If this finding for the dopamine D_2 receptor is generalizable, the variation in BP for 5-HT_{2A} receptors and SERT would reflect the interindividual variation in receptor/transporter densities. However, it cannot be excluded that the detected changes in receptor and transporter binding in the studies included in this thesis to some extent could be due to interindividual differences in K_d.

Altanserin-PET

A special concern with the use of ^{18}F-altanserin in humans is the systemic formation of lipophilic metabolites that do not bind to 5-HT_{2A} receptors. In our experimental set-up we determine the binding potential BP_P with the bolus-infusion approach developed by Pinborg et al (Pinborg, Adams 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, Lopresti et al. 2001) is that the contribution from radiolabeled lipophilic metabolites, can be subtracted directly, provided that a suitable reference region void of receptors exists.

According to *eq. 1,* the calculation of the outcome measure for 5-HT_{2A} receptor binding measurement, BP_p, relies on both the non-specific binding (distribution volume in cerebellum) and C_p. Thus, a relative underestimation of non-specific binding and/or C_P would lead to an overestimation of BP_P. In contrast, a high plasma free fraction of radiotracer, f_p, would lead to a high BP_p. When comparing the BP_p from subjects of two groups it is important to evaluate the influence of these parameters. In study 1, we detected a *positive* correlation between BMI and non-specific binding, which would tend to produce

results in the opposite direction of our results. This positive relationship was readily explained by the observation that a high BMI was associated with an increased plasma metabolite fraction. We also ruled out that there was any difference in f_p between normal-weighted and overweight/obese subjects in this study. Finally, no relationship was detected between BMI and C_p. In study 3, no difference was detected in non-specific binding or in f_p between schizophrenic patients and controls.

Our quantification of 5-HT_{2A} receptor BP_p is based on the assumption of a steady state condition of the radioligand in blood and brain tissue. In order to check for a possible difference in the steady state condition between normal weighted vs overweight/obsese subjects in study 1, the slope values of the lines combining the time points of the parent compound measurements in plasma were compared between the two groups for a random age-matched subsample of 11 obese vs 10 normal weighted subjects (Students t-test: BMI: 37±6 vs 23±2 kg/m^2, $p<0.001$; Age: 50±19 vs 54±12 years, $p=0.592$). No difference was detected indicating that the overweight/obese subjects suceeded equally well as lean subjects to attain constant plasma levels of the parent compound (Students t-test: Slope: 0.06±0.08 vs 0.07±0.05, $p=0.698$).

A lack of selectivity of a radioligand can complicate the interpretation of its binding measurements. The serotonin 2C receptor subtype is the receptor to which ^{18}F-altanserin has second highest affinity after the 2A (Tan, Baldwin et al. 1999). Since there is an abundant preclinical and clinical literature on the anorectic effects of 5-HT_{2C} agonism (De Vry and Schreiber 2000), it could be speculated if positive correlation between BMI and cortical ^{18}F-altanserin binding reflects changes in 2C rather than 2A serotonin receptor binding. However, this explanation is unlikely since ^{18}F-altanserin has been shown to have a 5-HT_{2A} vs 5-HT_{2C} receptor selectivity ratio of 20 (Tan, Baldwin et al. 1999), and in cerebral cortex the expression of 5-HT_{2A} is higher than 5-HT_{2C} receptors (Appel, Mitchell et al. 1990; Pompeiano, Palacios et al. 1994; Nichols and Nichols 2008). Moreover, we have previously shown in brain homogenate binding studies that blocking with the 5-$HT_{2B/2C}$ selective compound SB 206553 does not alter ^{18}F-altanserin binding (Kristiansen, Elfving et al. 2005). With regard to the dopamine D_2 receptor, a lower ratio of 5-HT_{2A} receptors to D_2 receptors in subcortical areas compared to cortical stresses the importance of using a selective ligand in the quantification of 5-HT_{2A} receptor binding. ^{18}F-altanserin has a 200 to 500 fold 5-HT_{2A} /D_2 selectivity measured as 1/(5-HT_{2A} Ki/D2 Ki) = 1/(0.13 to 0.3 nM/62nM) = 1/(0.002 to 0.005) (Tan, Baldwin et al. 1999; Kristiansen, Elfving et al. 2005), making it between 8 and 50 times more selective for the 5-HT_{2A} receptors than e.g.

^{18}F-setoperone, the radioligand used in most previous PET studies of the 5-HT_{2A} receptor in schizophrenia (Lewis, Kapur et al. 1999).

Evaluation of receptor binding with PET in a small region with relatively low 5-HT_{2A} receptor levels like the caudate nucleus should be performed with caution. In study 3, a preliminary finding of increased binding in this region was reported. No correction for multiple comparisons was made (14 regions were compared). Thus, such a finding should be confirmed in an independent sample.

It is known that antidepressant treatment with SSRIs can downregulate the 5-HT_{2A} receptors (Gray and Roth 2001; Benmansour, Owens et al. 2002). As four of the schizophrenic patients in study 3, had prior (n=3) or present (n=1) use of antidepressant medication, a post hoc analysis was therefore performed after removing these patients and their controls from the analysis. This did not change the outcome of the study.

DASB-PET

The use of a tissue-reference method has the advantage that it allows estimation of neuroreceptor/transporter binding potential without the invasive and logistically difficult procedure of obtaining arterial input functions corrected for metabolites. However, the use of such a method does not provide a regular estimate of non-specific binding of the radioligand. Since the BP_{ND} is relying on the uptake and washout of the ligand in the reference tissue, a difference between groups in non-specific binding would affect the BP_{ND}. As an alternative to a direct measure of the non-specific binding, the area under the reference region's time activity curve curves normalized to the injected dose can be evaluated. This was done in study 2 to rule out any cerebellar tracer uptake differences between the groups (normal weighted vs overweight/obese subjects). No such difference was detected.

MRI

If a difference between subjects included in a PET study is suspected in gray and white matter volumes or in the ratio of gray and white matter, a correction for partial volume effects (PVE) should be considered. The application of such a correction will counteract the loss of regional PET signal from a region due to spill out. Without PVE correction, subjects with volume reduced VOI will have their PET outcome underestimated as compared to controls. With age, gray matter decreases due to atrophy, so when investigating a group of subjects with a large age span, it is be meaningful to correct for

PVE. Further, it is especially important to account for the PVE in cortical areas where the gray matter tissue is surrounded by CSF. In study 3, partial volume correction ad modum Müller-Gartner was applied to the data.

However, the use of different MR scanners in the same study could potentially yield differences in the tissue segmentation and subsequently bias in the outcome measure, especially partial volume corrected outcome. In our laboratory, we have often chosen to conduct our data analysis both with and without partial volume correction. In some situations, such as in the comparison of patients with substantial brain atrophy and healthy controls, partial volume correction is necessary. Yet, at the same time partial volume correction is undoubtedly associated with the introduction of noise and therefore, the correction cannot be uniformly recommended in a sample of subjects without major structural brain abnormalities. In study 1, non-partial volume corrected data were chosen for the analysis and the MR type was included as a covariate. In paper 2, a region where the tissue structure does not allow for segmentation (midbrain) was included. Therefore, in order to allow comparability across regions, partial volume correction ad modum Müller-Gartner was not applied. To address the age related atrophy in both study 1 and 2 where a significant age span was present, age was included as a covariate in the analysis.

It is important to elucidate if changes in receptor/transporter binding are associated with differences in the amount of gray matter within the investigated VOIs. In imaging studies using computerized tomography or MRI, others have found that overweight and obesity was associated with decreased gray matter volume (Gustafson, Lissner et al. 2004; Pannacciulli, Del Parigi et al. 2006; Taki, Kinomura et al. 2008). To examine if the primary observations in study 1 and 2 were influenced by BMI-related differences in gray matter, we tested the association between BMI and the gray matter ratio in neocortex (study 1 and 2) and in the caudate-putamen-thalamus region (study 2) in a linear model. No relation between BMI and gray matter fraction was identified. In addition, no difference in gray matter volume was detected between schizophrenic patients and controls in any VOIs in study 3.

BMI

BMI as an indirect measure of body fat has some limitations. A highly trained person may have a BMI between 25 and 30 because of increased muscularity rather than increased body fatness. Furthermore, there are interracial differences in relation between body fat and BMI. However, BMI is still the most commonly used anthropometric estimate

of fatness for public health purposes. To find out whether BMI corresponded to another anthropometric estimate of the nutrition state of an individual, the waist circumference was obtained in a subsample of 36 subjects. Using normal linear regression, a positive relation between BMI and this measure was detected ($p<0.001$). Thus, the observation that BMI generally correlates well with other anthropometric measures such as waist circumference was confirmed (Molarius and Seidell 1998).

Results and discussion

The results are presented in detail in the papers/manuscripts covering study 1, 2, and 3. Here the findings are summarized and discussed. In addition to the papers/manuscripts, more details are given about the genotype and the comparison of pre- and postsynaptic markers.

BMI in relation to 5-HT_{2A} and SERT binding.

We found that cortical 5-HT_{2A} binding was significantly positively correlated to BMI (**fig 9**), whereas cortical and subcortical SERT was significantly negatively correlated to BMI (**fig 10**).

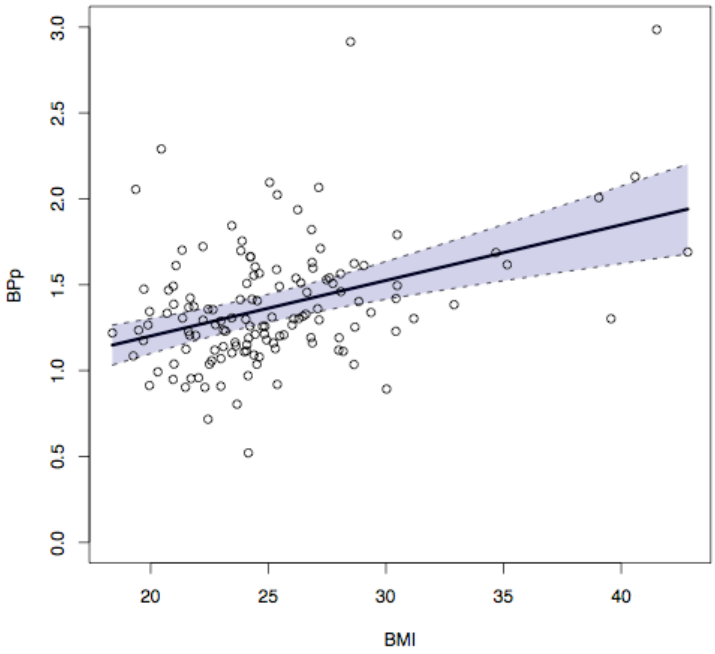


Figure 9

Plot of BMI vs. necortical 5-HT_{2A} receptor binding. The plotted BP_Pvalues are the partial residuals from the linear model with BMI, age, neuroticism and MR-type (intercept defined at mean neuroticism, mean age and 1.5T MR-type).

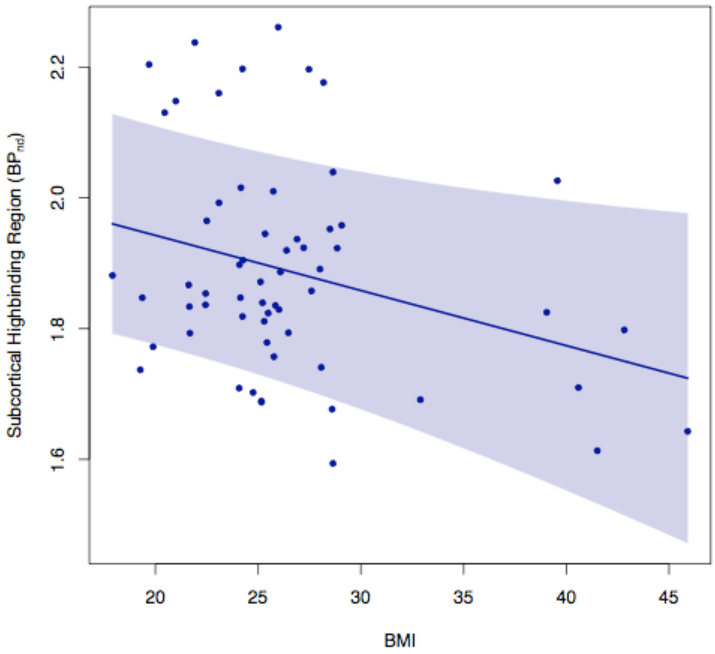


Figure 10
Plot of BMI vs. subcortical high-binding (caudatus-putamen-thalamus) SERT binding. The plotted BP_{ND} values are the partial residuals with 95% pointwise confidence limits from the linear model with BMI, age, and daylight minutes as (centered) co-variates.

In support of a positive relation between cerebral 5-HT_{2A} receptor and BMI, Huang et al recently demonstrated that mice that - when offered a high-fat diet - ate more and became obese displayed a higher cortical 5-HT_{2A} receptor density than their normal-weighted littermates on the same diet (Huang, Huang et al. 2004). In line with the demonstrated negative relationship between BMI and SERT binding in study 2, a similar negative correlation between BMI and cerebral SERT binding was also identified in a preliminary report by Matsumoto et al (Matsumoto 2008) based on 25 healthy human subjects. In addition, a relationship between impaired serotonergic transmission and binge eating behavior has been suggested by Kuikka et al who detected reduced midbrain SERT binding in binge eating obese women (Kuikka, Tammela et al. 2001). In contrast, twins with higher BMI were found to have higher SERT binding, as measured with ^{123}I-nor-β-CIT SPECT, than their monozygotic twin sibling with lower BMI (Koskela, Kaurijoki et al. 2008). In the latter study, BMI did not correlate with SERT binding in the entire group of 31 subjects (one subjects excluded from this analysis). The reason for the discrepancy between our and Matsumouto et al's results on one hand and Koskela et al's on the other, is not readily explained but the authors of the latter study (Koskela, Kaurijoki et al. 2008) suggest that ^{123}I-nor-β-CIT in contrast to other more thoroughly validated SERT ligands might be sensible to endogenous 5-HT levels. Although this issue has never been settled, then a reduced 5-HT would result in a higher SERT binding, as measured with ^{123}I-nor-β-CIT-SPECT.

Low levels of 5-HT?

The detected relationship between BMI and both SERT and 5-HT_{2A} receptor binding could reflect a direct role of these markers in regulation of appetite and food intake, or it could be secondary to other changes in the serotonergic neurotransmission. In this section, we address the possibility that synaptic 5-HT levels influence eating behaviour.

Both the high cerebral 5-HT_{2A} receptor binding and low SERT binding in subjects with high BMI could be the consequence of lower cerebral 5-HT levels in these subjects. Support of this notion comes from animal data where the effects on SERT and 5-HT_{2A} receptor binding following chronic changes in extracellular 5-HT levels have been investigated. As argued in the introduction section and illustrated in **fig 3**, these data suggest that a moderate decrease of endogenous 5-HT levels leads to an increase in 5-HT_{2A} receptor binding (Heal, Philpot et al. 1985; Cahir, Ardis et al. 2007) and a decrease in SERT binding (Rattray, Baldessari et al. 1996; Rothman, Jayanthi et al. 2003). This is agreement with our interpretation of low 5-HT levels as a possible explanation for the detected binding differences of the two investigated markers in overweight/obese subjects.

The model mentioned above is primarily based on experimental data but there are also human studies in support of the concept. Low cerebrospinal fluid levels of 5-HT metabolites have been found in women with primarily abdominal obesity (Bjorntorp 1995). Although chronic MDMA intake hardly can be regarded as a pure 5-HT depletion model, it is consistently reported that SERT binding is low in chronic MDMA users (Reneman, Endert et al. 2002; McCann, Szabo et al. 2005; Buchert, Thiele et al. 2007). Since MDMA after an acute phase of potent 5-HT release leads to a more chronic depletion of 5-HT (Morton 2005), these results could be interpreted as a down-regulation of SERT secondary to decreased brain levels of 5-HT in accordance with the above described animal literature. A rapid reversible reduction in cerebral 5-HT levels in humans by acute tryptophan depletion, however, does not alter cerebral SERT binding as measured with ^{11}C-DASB (Praschak-Rieder, Wilson et al. 2005; Talbot, Frankle et al. 2005). But since the protein trafficking and degradation is a more long-term process it is unlikely that acutely induced alterations in cerebral 5-HT levels alone would lead to immediate changes in SERT binding in the absence of competition effects. The observation that 5-HT depletion in an *in vitro* study did not decrease SERT binding until after 14 days supports this (Rattray, Baldessari et al. 1996).

Given that the cerebral 5-HT levels are low in overweight/obese subjects, it is worth

to consider the role of 5-HT in the regulation of body weight. The feeding pattern associated in a state with reduced cerebral 5-HT is altered towards both intake of smaller meals, slower eating but unchanged meal frequency (Blundell 1984; Leibowitz 1988; Simansky 1996), suggesting that 5-HT modulates satiety. It has been shown that serotonergic acting agents, especially when injected directly into the hypothalamus, suppress carbohydrate consumption while having little or no effect on the ingestion of protein or fat (Leibowitz and Alexander 1998). Likewise, carbohydrate ingestion leads to increased circulating levels of the 5-HT amino acid precursor, tryptophan (Fernstrom, Faller et al. 1975; Noach 1994; Wurtman and Wurtman 1995), as well as increased hypothalamic and raphe nuclei 5-HT (Leibowitz and Alexander 1998). Thus, 5-HT as a feedback on eating serves to terminate the meal and yield a state of satiety. As obese subjects tend to have a preference for carbohydrate rich food (Weltzin, Fernstrom et al. 1994; Wurtman and Wurtman 1995) it is possible that the 5-HT mediated feedback is disturbed in this condition. Interestingly, the per capita consumption of sugar has showed a 25% increase from 1970 to 2000 in the United States, reflecting a trend that has occurred in Western nations since the beginning of the industrial revolution 200 years ago (Cordain, Eaton et al. 2005). It could be speculated that people through a serotonergic hypofunction and consequently disturbed negative feedback mechanism could be more vulnerable to this increasingly carbohydrate-rich food.

Taken together, the physiology of normal eating behaviour seems to be related to serotonergic transmission and a serotonergic dysfunction seems to play an important part in the brain pathophysiology behind eating disorders through a disturbance of satiety mechanisms eventually leading to overeating and obesity. In line with this, our observations of a negative association between BMI and SERT binding as well as the positive association to 5-HT_{2A} receptor binding, could be interpreted as consequences of, or concomitant phenomena to low brain 5-HT levels in individuals with high BMIs.

A direct role of 5-HT_{2A} and SERT? Evidence from pharmacological and genetic studies.

As an alternative, the detected relationships between BMI and cerebral SERT and 5-HT_{2A} receptor binding could be due to shared genetic and/or early environmental factors directly affecting these two serotonergic markers.

In support of a specific role of the 5-HT_{2A} receptor in eating behaviour, Currie et al have in rats demonstrated that treatment with selective 5-HT_{2A} receptor antagonists - in

contrast to selective 5-HT_{2C} or 5-HT_{2B} antagonists - reverses the inhibitory effect of treatment with the mixed 5-$HT_{2A/C}$ agonist DOI (4-Iodo-2,5-dimethoxy-a-methylbenzeneethanamine hydrochloride) on neuropeptide Y induced hyperphagia (Currie, Coiro et al. 2002). However, another group has claimed that the specific 5-HT_{2A} receptor antagonist, MDL 100907, failed to block the hypophagia induced by DOI (De Vry and Schreiber 2000). The same authors further question the nature of DOI induced hypophagia as they claim that this effect is behaviourally non-specific when compared to the more specific enhancement of satiety in rats observed after selective 5-HT_{2C} or 5-HT_{1B} agonism. Thus, from pharmacological studies in animals, it is controversial whether 5-HT_{2A} receptor has a key role in the regulation of eating.

Genetic mechanisms are known to influence serotonin receptor levels and distribution. Pinborg et al (2007) has shown that the level and pattern of distribution of the 5-HT_{2A} receptor is tighter correlated in monozygotic twins than in dizygotic twins, and therefore is indeed heritable. Nevertheless, the 5-HT_{2A} receptor and SERT also respond to environmental factors, e.g. stress and high plasma cortisol (Tafet, Idoyaga-Vargas et al. 2001; Dwivedi, Mondal et al. 2005) and moreover, 5-HT_{2A} receptor is also modulated by postnatal levels of low brain derived neurotrophic factor (Rios, Lambe et al. 2006). Therefore, early environmental factors as well as adult life environment may also influence serotonergic neurotransmission critically.

A relationship between measures of overweight/obesity and specific genetic polymorphisms related to 5-HT_{2A} receptor and/or SERT expression would support that these markers could be directly involved in this condition - although such a relation in theory also be could mediated through effects on other components in the 5-HT neurotransmission rather than via direct regulation of the 5-HT_{2A} receptor and SERT levels. In support of a genetic component in the serotonergic involvement in regulation of bodyweight, SERT knockout mice, when aged approximately 3 months, become obese (Holmes, Murphy et al. 2002; Warden, Robling et al. 2005; Murphy and Lesch 2008). Additionally, an association between overweight/obesity and the S-allele of the SLC6A4 HTTLPR polymorphism has recently been demonstrated in both Argentinean adolescents (n=172) (Sookoian, Gemma et al. 2007) and in Hispanics and American white men (n=1584) (Fuemmeler, Agurs-Collins et al. 2008). Since evidence suggests that the S-allele confers decreased SERT expression and binding sites (Lesch, Bengel et al. 1996; Little, McLaughlin et al. 1998; Heinz, Jones et al. 2000), especially when taking triallelic variation in the 5-HTTLPR into account (Praschak-Rieder, Kennedy et al. 2007; Reimold,

Smolka et al. 2007) this is in good agreement with our finding. However, not all data support such a relation between 5-HTTLPR polymorphism and SERT density (Greenberg, Tolliver et al. 1999; Mann, Huang et al. 2000; Preuss, Soyka et al. 2000; van Dyck, Malison et al. 2004; Parsey, Hastings et al. 2006).

There are also studies that suggest a link between genes related to expression of 5-HT_{2A} receptors and body weight. In a meta-analysis of nine genetic studies of anorexia nervosa, the A-allele of the 5-HT_{2A} receptor G(-1438)A promoter polymorphism was shown to be associated with anorexia nervosa, although this vulnerability allele was suggested to be disorder modifying rather than causal (Gorwood, Kipman et al. 2003). The positive relation between the G-allele of the same polymorphism and body weight has also been established in studies of both adult overweight human subjects (Aubert, Betoulle et al. 2000; Sorli, Frances et al. 2008) (n=276 and 303, respectively) as well as in normal weighted adults (n=284) (Rosmond, Bouchard et al. 2002; Herbeth, Aubry et al. 2005). In a group of 370 children and adolescents, Herbeth et al did not see any relation between body weight and the G(-1438)A polymorphism but instead found that G-allele carriers displayed a higher energy and fat intake than the A carriers (Herbeth, Aubry et al. 2005). Together, data from these studies indicate that the expression of the 5-HT_{2A} gene could influence eating behavior in humans. However, it is possible that the association between the G(-1438)A polymorphism and food intake and/or body weight is more pronounced in subjects with a more extreme BMI, as suggested by Sorli et al (Sorli, Frances et al. 2008). This, could also explain the discrepancies seen in studies relating the G(-1438)A promoter polymorphism to anorexia nervosa. In a study of another 5-HT_{2A} receptor gene polymorphism, risperidone treatment of schizophrenic carriers of the T/T allel of the T102C single nucleotide polymorphism resulted in more body weight gain than the treatment did in the C/C allele carriers (Hong, Lin et al. 2001). Since postmortem levels of 5-HT_{2A} receptor mRNA levels have been shown to be higher in subjects with the T/T genotype (Polesskaya and Sokolov 2002), these data also support our finding of increased 5-HT_{2A} receptor binding in overweight subjects.

In study 1, no significant association was seen between neither the neocortical 5-HT_{2A} receptor binding and the A(-1438)G promoter polymorphism, nor between the 5-HT_{2A} receptor binding and the interaction between BMI and the A(-1438)G promoter polymorphism. Also, there was no differences in BMI within the 3 allelic groups (A/A, n=14; A/G, n=44; and G/G, n=37) (24.4±3.2, 25.0±3.3, and 24.4±3.3 kg/m^2 respectively). When A/A and A/G or A/G and G/G were pooled, there was still no between-group

difference in BMI (24.9±3.3 vs. 24.4±3.3, and 24.7±3.3 vs 24.4±3.2 kg/m^2).

With regard to the 5-HTT-linked polymorphic region (5-HTTLPR) investigated in 49 of the 60 ^{11}C-DASB scanned subjects, eighteen participants were homozygote L-allele carriers (37%), eleven were homozygote S-allele carriers (22%), and twenty heterozygotes (41%); these frequencies did not differ significantly (Pearson's Chi^2 test with one degree of freedom) from the frequencies expected according to the Hardy-Weinberg principle or expected according to the observed frequencies in a larger group of 847 Caucasian non-Maori participants (Caspi, Sugden et al. 2003). Among the total of eighteen homozygote L-allele carriers, fifteen were homozygote L_A-allele carriers, and three carried L_AL_G. No effect of being L_A-allele carrier was detected on either SERT binding in any of the three investigated VOIs (highbinding subcortical region: p=0.600, midbrain: p=0.942, neocortex: p=0.964), or on BMI (p=0.862). Neither was there any significant association between the SERT binding and the interaction between BMI and the 5-HTTLPR long (A/G) polymorphism.

Thus, earlier observations of a relation between BMI and either the G(-1438)A promoter polymorphism or the 5-HTTLPR long (A/G) polymorphism could not be confirmed in study 1 and 2. It should be pointed out that our sample sizes were smaller than in the other studies and only a relatively small proportion of the subjects in our studies were obese.

The relation of 5-HT_{2A} receptor and SERT binding to alcohol consumption and tobacco smoking.

Several lines of evidence have suggested an inverse relationship between alcohol intake and central serotonergic neurotransmission. Administration of 5-HT enhancing drugs reduce alcohol consumption in both animals and humans (Lyness and Smith 1992; Naranjo, Poulos et al. 1992; Sellers, Higgins et al. 1992; McBride, Bodart et al. 1995). Further, lower brain levels of 5-HT and its metabolites is seen in rats genetically selected for their preference to alcohol (Murphy, McBride et al. 1983; Zhou, Bledsoe et al. 1991; McBride, Bodart et al. 1995) and pharmacologically induced 5-HT depletion in rats increases their self-administration of intravenous alcohol (Lyness and Smith 1992). Decreased 5-HT_{2A} receptor binding in a number of different brain regions has also been detected in alcohol preferring rats (Ciccocioppo, Angeletti et al. 1999) and an enhanced 5-HT_{2A} receptor status has been detected in hypothalamus and corpus striatum (Akash, Balarama et al. 2008). Rodent studies have also shown that ethanol significantly elevates extracellular levels of 5-

HT (McBride, Murphy et al. 1993). From studies in humans, there is growing evidence of serotonergic alterations in alcoholism. In particular, reductions in SERT binding has been seen in the living and the postmortem brains of alcoholics (Heinz, Jones et al. 2000; Kranzler, Lappalainen et al. 2002). Furthermore, the 5-HT_{2A} receptor binding in frontal cortex was lower among subjects with than without a family history of alcoholism (Underwood, Mann et al. 2008), although no statistically significant difference was seen in cerebral 5-HT_{2A} receptor binding in a post mortem study of alcoholics and controls.

In our studies, no relationship between 5-HT_{2A} receptor or SERT binding and the degree of alcohol consumption was detected in non-alcoholic healthy subjects. We further tested if the finding by Underwood et al could be replicated with our data. Out of the 136 subjects in study 1, detailed information about family history of alcohol use was available for only 36 persons; 7 of these had at least one first degree relative with history of alcohol abuse, whereas the rest were undisposed. In this limited sample, we did not see any relation between familiar disposition to alcohol abuse on the 5-HT_{2A} receptor binding in neocortex and we were thus unable to reproduce their finding. It should be emphasized that the subjects in our studies consisted of healthy subjects with varying degree of alcohol intake and that subjects with alcoholism were excluded from the studies. It is possible that inclusion of heavy drinkers could have yielded a different result.

Observations parallel to those mentioned above for alcohol have been made for the relation between tobacco smoking and 5-HT neurotransmission. Thus, enhancement of serotonergic neurotransmission decreases nicotine intake (Opitz and Weischer 1988), whereas repeated nicotine treatment decreases frontocortical 5-HT levels in animals (Harrison, Everitt et al. 1997; Olausson, Engel et al. 2002). In line with the latter observation, tobacco constituents can raise brain 5-HT levels by the blocking effect on monoamone oxidase (MAO) activity (van Amsterdam, Talhout et al. 2006). Used in combination with transdermal nicotine substitution therapy, SSRI has furthermore been shown to increase nicotine abstinence symptoms over placebo (Killen, Fortmann et al. 2000) even though a central role of SSRI treatment for smoking cessation has recently been questioned in Cochrane review (Hughes, Stead et al. 2007). We used two different approaches to test if tobacco smoking among the subjects in our studies was related to SERT and/or 5-HT_{2A} receptor binding; a dose-response analysis and “effect of ever smoked vs. never smoked” analysis. We did not detect any relationship using these two approaches between tobacco smoking and the binding potentials of any of the two markers. It should be noted that our studies were not directly designed to examine the effect of smoking and

therefore relatively few tobacco smokers, especially in study2 (n=12), were included in the study. Accordingly, we cannot exclude that an effect of smoking could be demonstrated in a study with sufficient power to study this specific question.

Combination of the two marker; the relationship between 5-HT_{2A} receptor and SERT binding (aim 4)

From evaluation of the estimated functional form of the data from the explorative non-parametric analysis, an inverted U-shaped relationship between SERT and 5-HT2A receptor binding seemed reasonal. Based on AIC-values for each of the three investigated SERT region with different degrees of polynomials (data not shown), a quadratic expression was sufficient for all three regional relationships. The estimated quadratic are presented in **fig 11**. The second order term is significant (p=0.001) with a p-value for an overall test for no association between the two measurements given by 0.004). Dividing the sample into two group of subjects with time intervals between the two PET measurements of less or more than two weeks did not change the results; no difference between these two groups was detected.

A)

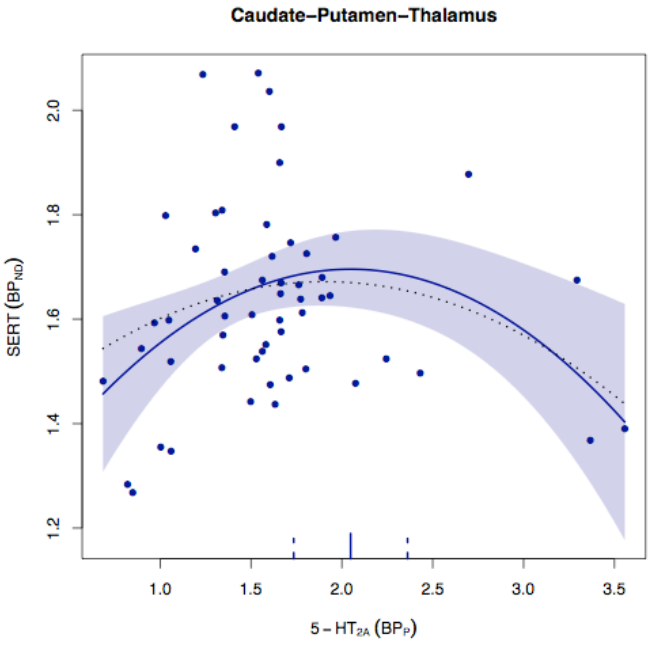
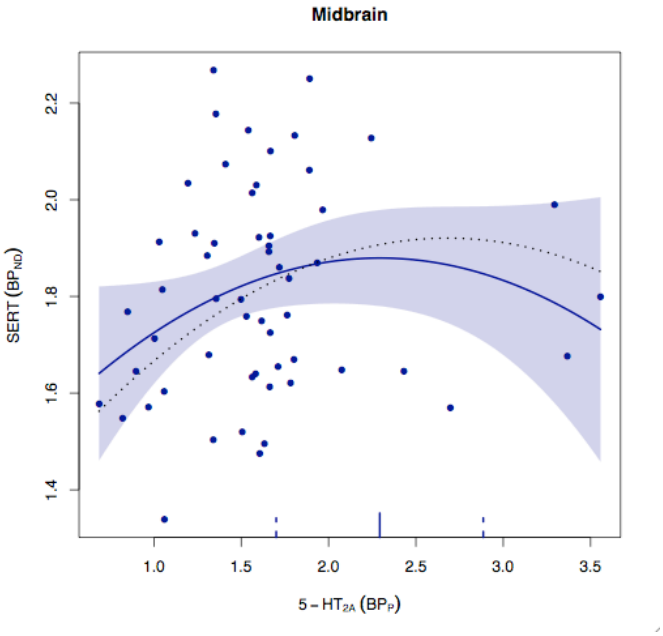


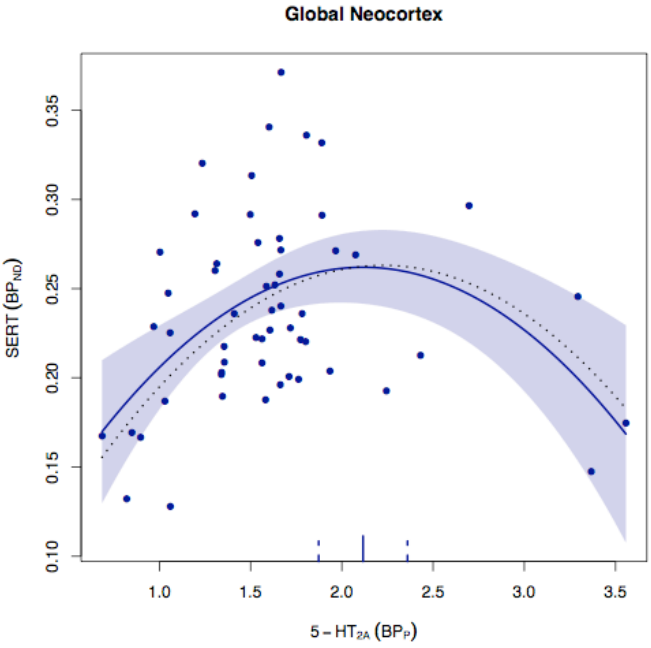
Figure 11

The estimated quadratic association with pointwise 95% confidence bands and toppoints of the curves with approximate 95% confidence limits are presented for neocortical 5-HT_{2A} BP_p vs regional SERT BP_{ND} . The Y-axis represents SERT BP_{ND} in the subcortical high-binding region (caudate-putamen-thalamus) (A), midbrain (B), and neocortex (C), respectively.

B)


Midbrain
SERT (BP_{ND})
1.4
1.6
1.8
2.0
2.2
1.0
1.5
2.0
2.5
3.0
3.5
5 – HT_{2A} (BP_P)

C)


Global Neocortex
SERT (BP_{ND})
0.10
0.15
0.20
0.25
0.30
0.35
1.0
1.5
2.0
2.5
3.0
3.5
5 – HT_{2A} (BP_P)

As reviewed in the Introduction, there is evidence from the animal literature in support of a relationship between synaptic 5-HT levels and 5-HT_{2A} receptor binding. We have previously observed that a certain extent of "autocorrelation" between regional levels of the 5-HT_{2A} receptor binding within the single individual exist (Erritzoe 2007), and this has been interpreted as a regulation that takes place according to interindividual differences in central 5-HT levels. This interpretation has also been employed for data from *in vivo* 5-HT_{2A} receptor studies of Tourettes (Haugbol, Pinborg et al. 2007), mood disorders (Meyer 2007), and MCI (Hasselbalch, Madsen et al. 2008). Recognizing 5-HT_{2A} binding density as a surrogate marker of synaptic 5-HT levels, we accordingly have demonstrated a positive relation between "5-HT levels" and SERT binding for subjects with low 5-HT levels, and an inverse relation with SERT binding was observed for subjects with high 5-HT levels. This inverted U-shaped relation between "5-HT levels" and SERT binding agrees well with experimental studies where both 5-HT de- and increasing manipulations leads to decreased SERT binding (Pineyro, Blier et al. 1994; Rattray, Baldessari et al. 1996; Benmansour, Cecchi et al. 1999; Horschitz, Hummerich et al. 2001; Benmansour, Owens et al. 2002; Gould, Pardon et al. 2003; Rothman, Jayanthi et al. 2003; Gould, Altamirano et al. 2006).

In addition to these 5-HT manipulation studies, there are complementary evidence in support of a relationship between SERT and 5-HT_{2A} receptor binding. For example, depending on the used radioligand SERT knock-out mice have 5-HT_{2A} receptor density changes that go in different directions; when measured with an antagonist radioligand, a global decrease in 5-HT_{2A} binding is seen (Rioux, Fabre et al. 1999) whereas when measured with an agonist radioligand, a decreased binding is found in striatum, an increased binding in hypothalamus and no change in cortex (Li, Wichems et al. 2003). This difference could be explained by a regionally changed ratio of high and low affinity states of the 5-HT_{2A} receptors in knock-out mice.

Interestingly, these serotonin transporter knock-out mice having six-fold elevated extracellular 5-HT levels (Bengel, Murphy et al. 1998; Montanez, Owens et al. 2003; Mathews, Fedele et al. 2004) also have reduced 5-HT_{2A} receptor mediated serotonergic signaling (Qu, Villacreses et al. 2005). Similarly, chronic blockade of SERT decreases 5-HT_{2A} receptor responsiveness (Maj and Moryl 1993; Kennett, Lightowler et al. 1994; Yamauchi, Miyara et al. 2006) whereas mice that over-express SERT, when stimulated with a 5-HT_{2A} receptor agonist, show increased specific 5-HT_{2A} receptor behavioral response (Jennings, Sheward et al. 2008).

In summary, the detected relationship between SERT and 5-HT_{2A} receptor binding suggest a common regulation of these markers. We propose that the observed non-linear relation is secondary to inter-individual differences in synaptic 5-HT levels although other factors, such as disease effects, also are likely to play a role in the the expression of the two markers.

5-HT_{2A} receptor binding in neuroleptic-naïve schizophrenic patients

So far, the number of neuroleptic-naive schizophrenic patients enrolled in our study (study 3) is the largest sample examined in an *in vivo* imaging study of the 5-HT_{2A} receptor in schizophrenia. Earlier studies have reported on 6 (Ngan, Yatham et al. 2000), 7 (Trichard, Paillere-Martinot et al. 1998), 10 (Okubo, Suhara et al. 2000) and 10 (Lewis, Kapur et al. 1999) schizophrenic patients, respectively. In agreement with 3 out of 4 of these previous PET studies (Trichard, Paillere-Martinot et al. 1998; Lewis, Kapur et al. 1999; Okubo, Suhara et al. 2000), we did not observe any difference in cortical 5-HT_{2A} receptor BP_P between 15 schizophrenic patients and 15 age-gender-BMI matched controls. In contrast, Ngan et al. (Ngan, Yatham et al. 2000) reported a decreased 5-HT_{2A} binding in frontal cortex of six neuroleptic-naïve schizophrenic subjects and such a difference was also observed in a recent PET study of 6 subjects with elevated risk of developing schizophrenia (Hurlemann, Boy et al. 2005).

Despite some inconsistency in postmortem data, the majority of post-mortem studies suggest a decreased cortical 5-HT_{2A} receptor binding in patients with schizophrenia. This is in contrast to the outcome of most other in vivo studies, including our own. This discrepancy might be caused by the influence of antipsychotic drug treatment and cause of death in the studies of postmortem brain tissues. Suicide as a cause of death has been associated with increased postmortem 5-HT_{2A} receptor density, especially in younger cohorts (Oquendo, Russo et al. 2006). At the same time treatment with antipsychotic drugs that antagonize the 5-HT_{2A} receptor decreases levels of expression of the receptor (Mikuni and Meltzer 1984; Andree, Mikini et al. 1986; Milburn 1989; Wilmot CA 1989; O'Dell, La Hoste et al. 1990; Dean 2003). These factors need to be taken into account when interpreting reports of 5-HT_{2A} receptor levels in postmortem tissue from schizophrenic patients. In vivo PET data from antipsychotic-naïve patients are spared from such confounders and might be more reliable in assessing receptor regulations in schizophrenia. On the other hand, autoradiographic post mortem studies do - in contrast to PET imaging - allow for detection of differences in receptor binding within cortical cell layers.

We detected an increased 5-HT_{2A} receptor binding in the caudate nucleus in the patient group as compared to the controls (**table 2**). No significant relationships between caudate 5-HT_{2A} receptor binding and positive, negative, or general PANSS scores were detected. The 5-HT_{2A} receptor density in subcortical brain regions is only modest yielding a low signal to noise ratio. For these reasons, and since no corrections for multiple comparisons were made, we consider the finding of an increased 5-HT_{2A} receptor binding in the caudate nucleus in schizophrenic patients as preliminary.

In conclusion, further studies much be conducted to replicate the findings on 5-HT_{2A} receptor binding in patients with schizophrenia.

Table 2:

Partial volume corrected binding potentials of the specific ^{18}F-altanserin binding (BP_P) in regions of interest of schizophrenic patients and controls, respectively.

Region	**Schizophrenic Patients**	**Control Subjects**	**P-value**
Neocortex	2.8 ± 0.6	2.7 ± 0.9	NS (0.60)
Frontal Ctx (total)	2.9 ± 0.6	2.7 ± 0.9	NS (0.51)
Orbitofrontal Ctx	2.7 ± 0.6	2.5 ± 0.8	NS (0.44)
Medial Inf Frontal Ctx	3.0 ± 0.6	2.8 ± 0.9	NS (0.52)
Superior Frontal Ctx	3.0 ± 0.6	2.8 ± 0.9	NS (0.54)
Anterior Cingulate	2.4 ± 0.5	2.2 ± 0.8	NS (0.48)
Posterior Cingulate	2.6 ± 0.6	2.3 ± 0.7	NS (0.36)
Insula	2.0 ± 0.4	1.9 ± 0.6	NS (0.47)
Medial Inf Temp Ctx	2.6 ± 0.6	2.5 ± 0.8	NS (0.55)
Sup Temp Ctx	2.7 ± 0.5	2.5 ± 0.8	NS (0.45)
Parietal Ctx	3.3 ± 0.7	3.1 ± 1.0	NS (0.63)
senmotc_av	2.8 ± 0.6	2.6 ± 0.8	NS (0.43)
Occipital Ctx	2.8 ± 0.6	2.9 ± 0.9	NS (0.75)
Thalamus	0.6 ± 0.1	0.5 ± 0.2	NS (0.15)
Putamen	0.7 ± 0.2	0.6 ± 0.3	NS (0.37)
Caudate	0.7 ± 0.1	0.5 ± 0.3	0.02

Conclusions

Based on *in vivo* PET-measurements in humans, we have identified a characteristic pattern in the pre- and postsynaptic serotonergic markers that may strengthen the understanding of the neurobiology behind regulation of body weight. The observation of globally decreased cerebral SERT and increased cerebral 5-HT_{2A} receptor binding in subjects with high BMI could be secondary to – and thereby a surrogate marker of - low 5-HT levels. Low synaptic 5-HT levels have been shown to lead to both a compensatory upregulation of cerebral 5-HT_{2A} receptor binding and to a downregulation of SERT. Also, hypofunction of serotonergic neurotransmission results in increased appetite and food intake and thereby to overweight and obesity. Particularly, with regard to carbohydrate intake, serotonergic hypofunction seems to be linked to a disturbed satiety mechanism. This might partly explain the increasing prevalence of obesity among citizens in, especially, Western countries, where a general change in food patterns towards an increased consumption of carbohydrate-rich food is seen.

However, rather than being a reflection of low 5-HT levels, it can not be excluded that the observation of low SERT and high 5-HT_{2A} in relation to high BMI could be due to more direct involvement of these two markers in the regulation of body weight. By understanding better the physiology of regulation of body weight, our findings may contribute to develop better treatment for eating disorders and obesity, and maybe even preventive strategies for persons at risk for developing eating disorders/obesity.

Both alcohol consumption and tobacco smoking was not related to 5-HT_{2A} receptor and SERT binding in healthy non-abusing individuals.

Furthermore, we detected a non-linear relationship between 5-HT_{2A} receptor and SERT binding in a large sample of subjects scanned with both markers. The observed inverted U-shaped relation is in agreement with experimental studies of how these markers conform to differences in endogenous 5-HT levels. Our demonstration of a differential regulation of the markers underpins the added information by conducting simultaneous measurements of 5-HT_{2A} receptor and SERT.

Finally, in line with most other *in vivo* imaging studies, we detected no between-group difference in cortical 5-HT_{2A} receptor binding in our study of 15 neuroleptic-naive schizophrenic patients and 15 age-, gender- and BMI-matched healthy controls.

Interestingly, increased 5-HT_{2A} receptor binding was seen in the caudate nucleus in the group of patients. However, this finding is regarded as preliminary and should be replicated.

Research perspectives

A number of new experiments could be interesting to follow-up on the results presented in this thesis.

- Addressing the causality of the relation between BMI and 2A/SERT would be of interest. Do changes in the serotonin system cause changes in eating behaviour and consequently changes in body weight which is the most common perception, or does food intake and/or body weight affect the serotonergic transmission which is supported by some reports? In this regard, the following studies in humans could be of interest:
    - Longitudinal design with exploration of whether weight loss or weight gain is associated with changes in of pre- and postsynaptic markers the cerebral 5-HT system using PET imaging (e.g. SERT and 5-HT_{2A}).
    - Monitoring of voluntary food intake in individuals with equal BMI in relation to the above mentioned markers of the cerebral 5-HT system. This would allow to test for associations between individual preferences for particular food components and markers of serotonergic neurotransmission.
- In humans with regular recreational MDMA use: Same measures as in 1. This study could serve as a model of long term 5-HT depletion in addition to being a regular study of long term effects of MDMA use. If our interpretation of the findings with BMI are correct, we hypothesize that MDMA users will have a change in serotonergic markers similar to that observed in overweight/obese subjects.
- In schizophrenic patients: Evaluation of 5-HT_{2A} receptor binding in a larger cohort, and use of new ligand (see below) more suited for 5-HT_{2A} receptor imaging in subcortical brain areas could shed light on the possible involvement of subcortical 5-HT_{2A} receptors in the pathophysiology of schizophrenia.

Methodological developments that would have great impact on the possibility to study the *in vivo* dynamics of serotonergic neurotransmission in humans include:

- Development of new high-affinity radioligands potentially suited for:
    - imaging SERT binding in all cortical regions and serotonin 2A receptor binding in subcortical regions.
    - measurements of endogenous serotonin release.

- Development of tracers for the imaging of new targets, e.g. the serotonin 2C and 4 receptors. The 5-HT_{2C} is strongly suspected to be critically and directly involved in eating behavior mainly mainly through hypothalamic pathways. It would be of great importance to investigate the receptor density in obese human subjects. In autoradiographical studies with 5-HT manipulation in rat, the 5-HT_4 receptor has been shown be positively correlated to 5-HT levels (Licht, submitted). Thus, adding measurements of the 5-HT_4 receptor could add to the indirect characterization of synaptic 5-HT levels.
- Implementation of high resolution PET, allowing valid evaluation of smaller brain regions such as the hypothalamus.
- Alternative methods for evaluation of endogenous levels of serotonin *in vivo* in humans, ideally non-invasive.

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Brain serotonin 2A receptor binding: Relations to body mass index, tobacco and alcohol use

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ABSTRACT

Manipulations of the serotonin levels in the brain can affect impulsive behavior and influence our reactivity to conditioned reinforcers. Eating, tobacco smoking, and alcohol consumption are reinforcers that are influenced by serotonergic neurotransmission; serotonergic hypofunction leads to increased food and alcohol intake, and conversely, stimulation of the serotonergic system induces weight reduction and decreased food/alcohol intake as well as tobacco smoking. To investigate whether body weight, alcohol intake and tobacco smoking were related to the regulation of the cerebral serotonin 2A receptor (5-HT_{2A}) in humans, we tested in 136 healthy human subjects if body mass index (BMI), degree of alcohol consumption and tobacco smoking was associated to the cerebral in vivo 5-HT_{2A} receptor binding as measured with ^{18}F-altanserin PET. The subjects' BMI's ranged from 18.4 to 42.8 (25.2 ± 4.3) kg/m^2. Cerebral cortex 5-HT_{2A} binding was significantly positively correlated to BMI, whereas no association between cortical 5-HT_{2A} receptor binding and alcohol or tobacco use was detected. We suggest that our observation is driven by a lower central 5-HT level in overweight people, leading both to increased food intake and to a compensatory upregulation of cerebral 5-HT_{2A} receptor density.

Introduction

Serotonergic neurotransmission in the brain is involved in the inhibitory control of behavior. Evidence for this notion comes from different lines of research as reviewed below. Depletion of the monoamine neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) in animals consistently leads to an impulsive behavioral pattern with increased responding for conditioned reinforcers (Fletcher, 1996; Fletcher et al., 1999; Sills et al., 1999) and manipulations that decrease brain 5-HT neurotransmission have been shown to elevate self-administration of food (Saller and Stricker, 1976; Waldbillig et al., 1981) and alcohol (Lyness and Smith 1992; Ciccocioppo, 1999; Ciccocioppo et al., 1999) as well as the attentional salience of tobacco smoking (Hitsman et al., 2007). On the other hand, manipulations that increase 5-HT levels (eg by administrating SSRI) inhibit intake of both food, alcohol, and nicotine in both animals and humans (Olausson et al., 2002; Halford et al., 2005; Johnson, 2008).

Overweight and obesity are conditions defined as abnormal or excessive accumulation of fat that may impair health. According to the latest data from World Health Organization (WHO 2006), on a global scale approximately 1.6 billion adults are overweight, and at least 400 million of them are obese. Body mass index (BMI), defined as the weight in kilograms divided by the square of the height in meters (kg/m^2), is used as a convenient measure for the nutrition state of an individual and is known to generally correlate well with other anthropometric measures such as waist circumference (Molarius and Seidell, 1998). BMI of more than 25 kg/m^2 is defined as overweight, and a BMI over 30 kg/m^2 as obese. Overweight is an important risk factor for developing a variety of disorders, including type-2 diabetes, cardio-vascular diseases, and certain types of cancer.

The involvement of 5-HT in the regulation of food intake and body weight is well established: Administration of agents that are either

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toxic to 5-HT neurons (e.g. 5,7-dihydroxytryptamine, 5,7-DHT) or prevent 5-HT synthesis (e.g. parachlorophenylanine, pCPA) increase food intake in rats with a subsequent increase in body weight (Saller and Stricker 1976; Waldbillig et al. 1981). Conversely, increased central 5-HT levels following administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP) or of the 5-HT releasing agent fenfluramine significantly decrease food intake (Clineschmidt 1973; Barrett and McSharry, 1975; Blundell and Leshem, 1975; Duhault et al., 1975). Both experimentally and clinically, administration of the 5-HT releasing agent fenfluramine, the selective serotonin reuptake inhibitor (SSRI) fluoxetine, the serotonin and norepinephrine reuptake inhibitor (SNRI) sibutramine, the 5-$HT_{1B/2C}$ agonist mCPP, and the 5-$HT_{1B/1D}$ agonist sumatriptan all lead to a reduced food intake and subsequent weight loss (Halford et al., 2005). In further support of a central role of serotonin in the regulation of food intake, selective activation of 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors leads to hypophagia in various animal models (Dourish, 1995; Bickerdike et al., 1999).

Different lines of evidence suggest that the 5-HT_{2A} receptor has a specific role in the regulation of body weight. G/G carriers of the A(−1438)G promoter polymorphism of the 5-HT_{2A} receptor gene have increased body mass and predominantly abdominal distribution of body fat (Rosmond et al., 2002). Some studies argue that A carriers of the same polymorphism are at higher risk of developing anorexia nervosa but these results are more mixed (Ricca et al., 2002). In vivo imaging studies using Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) have suggested that both anorexia nervosa patients and patients recovered from regular anorexia nervosa and bulimia-type anorexia nervosa display decreased cerebral 5-HT_{2A} receptor binding (Kaye et al., 2005). Moreover, obesity-prone mice exposed to high-fat diet were found to have increased 5-$HT_{2A/2C}$ receptor density in comparison to obesity-resistant mice fed on the same diet (Huang et al., 2004). Finally, in a sample of 52 healthy, largely normal-weighted subjects, we have previously identified a positive correlation between BMI and 5-HT_{2A} receptor binding in the left superior temporal cortex, left medial inferior temporal cortex, right dorsal lateral prefrontal cortex, and right sensory motor cortex ($p<0.0125$) (Adams et al., 2004). We now investigated if this serendipitous observation could be replicated in a larger, independent sample including overweight and obese people.

The purpose of the present study was to test in healthy human subjects whether BMI, alcohol consumption, and tobacco smoking were associated with changes in the cerebral 5-HT_{2A} receptor binding using ^{18}F-altanserin-PET. We also explored possible associations between the 5-HT_{2A} A(−1438)G promoter polymorphism and BMI.

Methods and materials

Participants and interviews

We included 136 adult human subjects (51 females, with a mean age of 40.5 ± 18.6 years, and a mean BMI of 25.2 ± 4.3 kg/m^2) in the study. Fourteen of the subjects had BMI above 30 and were thus classified as obese.

Written informed consent was obtained according to the declaration of Helsinki II, and the study had been approved by the Copenhagen 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, (KF) 12-142/03, (KF) 01-124/04, (KF) 01-156/04, and (KF) 01-2006-20).

Fifty-two of the healthy subjects were reported in Adams et al. (2004). The cohort of the remaining 84 subjects was collected in the period from 2003 to 2008, to address the issue of BMI (ensuring inclusion of overweight people), and for subsets to serve as non-obese control subjects in other ongoing studies of Tourette's (Haugbol et al., 2007a,b), schizophrenia (Erritzoe et al., 2008), and mild cognitive impairment (Hasselbalch et al., 2008). Subsets of the cohort have been reported in ^{18}F-altanserin reproducibility studies (Haugbol et al., 2007a,b; Marner et al., 2008) and in a study on the association between neuroticism and 5-HT_{2A} binding (Frokjaer et al., 2008).

None of the subjects had stimulant abuse or history of neurological or psychiatric disorders. All subjects were naive for antipsychotics and antidepressants, and all had a normal neurological examination on the day of the PET scan. On the day of the PET scan subjects completed the Symptom Check List Revised (SCL-90-R) questionnaire in order to assess symptoms of distress and psychopathology (Derogatis, 1994). The Danish version of the 240-item NEOPI-R self-report personality questionnaire (Hansen and Mortensen, 2004) was also filled in by the subjects on the day of the PET scan. The NEO-PI-R evaluates the broad personality dimensions of neuroticism, extraversion, openness, agreeableness, and conscientiousness.

Subjects were interviewed about tobacco smoking habits and use of alcohol using an in-house made questionnaire (The Copenhagen Alcohol and Smoking Questionnaire). Data about smoking habits were available for 131 of the 136 subjects. Based on the amount of tobacco use the subjects were divided into five categories: 1) subjects who had never smoked tobacco ($n=83$); 2) previous tobacco-smokers with no current use ($n=12$); 3) light smokers (1 to 4 cigarettes per day, $n=10$); 4) intermediate smokers (5 to 14 cigarettes per day, $n=12$); 5) heavy smokers (15 or more cigarettes per day, $n=14$). For a dose dependency analysis of effects of tobacco use, only categories 1 and 3–5 were included. In order to be able to address the effect of smoking vs. non-smoking, categories 1 and 2 were pooled into one group of subjects without present use of tobacco and compared to the smokers (categories 3–5 together). Finally, to explore the effect of "ever used tobacco" vs. "never used tobacco", categories 2–5 were pooled and compared to category 1.

Regarding alcohol consumption the subjects were divided into the following 4 categories: 1) subjects who drank maximum 2 units of alcohol per week ($n=34$); 2) subjects who drank 3 to 9 units of alcohol per week ($n=54$); 3) subjects who drank 10 to 21 units of alcohol per week ($n=33$); 4) subjects who drank more than 21 units of alcohol per week ($n=4$).

5-HT_{2A} receptor G(−1438)A promoter polymorphism

Ninety-five subjects were genotyped for determination of the 5-HT_{2A} receptor gene G(−1438)A (rs6311) promoter polymorphism. Genomic DNA was extracted from whole blood, specifically buffy coat lymphocytes, using a purification set from Qiagen Incorporate (www.qiagen.com).

The 5-HT_{2A} G(−1438)A promoter polymorphism was identified by using the PCR protocol described by Masellis et al. (1998). In short, PCR amplification of a 200 base pairs (bp) fragment was generated by forward primer 5′-CTA GCC ACC CTG AGC CTA TG-3′ and reverse primer 5′-TTG TGC AGA TTC CCA TTA AGG-3′ and followed by restriction enzyme digestion with MspI for 4 h. PCR fragment were separated on a 2% agarose gel (SeaKem GTG Agarose, www.cambrex.com). An A at position −1438 leads to an uncut fragment of 200 bp and a G at position −1438 leads to two fragments of length 121 bp and 79 bp.

^{18}F-altanserin radiosynthesis and administration

The radiosynthesis of ^{18}F-altanserin was prepared according to a previously described method by Lemaire et al. (1991). Quality control was performed using analytical thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The absence of residual solvents (methanol, THF and DMSO) was confirmed by ^{1}H NMR. For each PET study, 0.3–3.5 GBq of ^{18}F-altanserin was produced with a radiochemical yield greater than 95% and a mean specific activity of 63.8 ± 39.4 GBq/μmol. Catheters were

inserted in both cubital veins for tracer infusion and blood sampling, respectively. ^{18}F-altanserin was administrated as a combination of a bolus injection followed by continuous infusion to obtain steady state of the tracer in blood and tissue. The bolus-infusion ratio was 1.75 h, as previously described (Pinborg et al., 2003). Subjects received a maximum dose of 3.7 MBq/kg bodyweight ^{18}F-altanserin. The amount of injected cold altanserin in nmol/kg bodyweight was calculated as (injected dose/specific activity)/bodyweight) for the 92 (50 normal-weighted and 42 overweight/obese) subjects where both parameters were available.

Imaging and blood sampling

PET scans were acquired in tracer steady state conditions with an eighteen-ring GE-Advance scanner (GE, Milwaukee, Wisconsin, USA) operating in 3D-acquisition mode, producing 35 image slices with an interslice distance of 4.25 mm. The total axial field of view was 15.2 cm with an approximate in-plane resolution of down to 5 mm. Reconstruction, attenuation and scatter correction procedures were conducted according to DeGrado et al. (1994).

Ninety minutes after bolus injection of ^{18}F-altanserin, the subjects were placed in the scanner. Subjects were aligned in the scanner using a laser system so that the detectors were parallel to the orbito-meatal line, and positioned to include the cerebellum in the field of view using a short 2 min transmission scan. All subjects were scanned in a resting state. A 10-min transmission scan was obtained for correction of tissue attenuation, using retractable ^{68}Ge/^{68}Ga pin sources. The transmission scans were corrected for tracer activity by a 5-min emission scan performed in 2D mode. Dynamic 3D emission scans (5 frames of 8 min) started 120 min after tracer administration.

Data were reconstructed into a sequence of 128*128*35 voxel matrices, each voxel measuring 2.0*2.0*4.25 mm, with software provided by the manufacturer. A 3D re-projection algorithm with a transaxial Hann filter (6 mm) and an axial ramp filter (8.5 mm) was applied. Corrections for dead-time, attenuation, and scatter were performed.

Because the magnetic resonance (MR) scanner was exchanged in the period between 2003 and 2008, structural brain imaging was conducted using either a 1.5 Tesla Vision ($n=67$) or a 3 Tesla Trio scanner ($n=69$) (Siemens, Erlangen, Germany), the latter using an eight-channel head coil (In vivo, FL, USA). All subjects underwent high-resolution 3D T1-weighted, sagittal, magnetization prepared rapid gradient echo (MPRAGE) scans of the whole head (1.5 T: $1.2\times1.2\times1.1$ mm voxels, 158 slices; 3 T: $1\times1\times1$ mm voxels and 192 slices). Moreover, on the 3 Tesla scanner whole brain T2 weighted images were acquired. MPRAGE images were segmented into gray matter, white matter, and cerebrospinal fluid tissue classes using SPM2 (Wellcome Department of Cognitive Neurology, London, UK) to enable partial volume correction of the PET data. All 3 Tesla MR-images were corrected for spatial distortions due to non-linearity in the gradient system of the scanner (Jovicich et al., 2006) using the gradient non-linearity distortion correction software distributed by the Biomedical Informatics Research Network (http://www.nbirn.net) and for RF-inhomogeneities using the N3 software (Sled et al., 1998). Finally, for 3 Tesla scans tissue probability images were cleaned for extracerebral tissue using an automatically created brain mask based on T2 images while manual edited brain masks were used for 1.5 Tesla images.

Blood samples

Five venous blood samples were drawn at mid-scan times 4, 12, 20, 28 and 36 min after starting the dynamic scanning sequence. The samples were immediately centrifuged, and 0.5 ml of plasma was counted in a well counter for determination of radioactivity. Three of the 5 blood samples drawn at 4, 20, and 36 min were also analyzed for percentage of both parent compound (^{18}F-altanserin) and the metabolized radiotracer using reverse-phase HPLC following the procedure described by Pinborg et al. (2003).

In addition, the free fraction of ^{18}F-altanserin in plasma, f_P, was estimated using equilibrium dialysis, following a modified procedure by Videbaek et al. (1993). The dialysis was performed using Teflon-coated dialysis chambers (Harvard bioscience, Amika, Holliston, USA) with a cellulose membrane that retains proteins with a molecular weight >10,000 Da. A small amount of ^{18}F-altanserin (approximately 1 Mbq) was added to 10 ml plasma samples drawn from the subjects. 500 µl of plasma was then dialyzed at 37 °C for 3 h against an equal volume of buffer, since pilot studies had shown that 3 h equilibration time yielded stable values. The buffer consisted of 135 mM NaCl, 3.0 mM Kcl, 1.2 nM $CaCl_2$, 1.0 mM $MgCl_2$, and 2.0 mM phosphate (pH 7.4). After the dialysis, 400 µl of plasma and buffer were counted in a well counter, and f_P of ^{18}F-altanserin was calculated as the ratio of $DPM_{buffer}/DPM_{plasma}$ (DPM = disintegrations per min).

Data analysis

MR/PET co-registration

PET and MR images were co-registered using a Matlab (Mathworks Inc., Natick, MA, USA) based program (Willendrup et al., 2004) where PET and MR images are brought to fit through manual translation and rotation of the PET image with subsequent visual inspection in three planes (Adams et al., 2004).

Volumes of interest (VOIs)

VOIs were automatically delineated on each individual's transaxial MRI slices in a strictly user-independent fashion (Svarer et al., 2005). With this approach, a template set of 10 MRIs is automatically co-registered to a new subject's MRI. The identified transformation parameters are used to define VOIs in the new subject MRI space and through the co-registering these VOIs are transferred onto the PET images.

A global neocortical region was defined for each subject and served as the primary region of interest. This region consisted of a volume-weighted average of 8 cortical regions (orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, superior temporal cortex, medial inferior temporal cortex, sensory motor cortex, parietal cortex, and occipital cortex). Insula, hippocampus, anterior and posterior cingulate were also defined and served as regions of interest in a post-hoc analysis. The cerebellum was defined and used for non-specific binding measurements (see below). Within each volume of interest, the ratio between gray matter volume and the sum of the white plus gray matter volumes was computed.

Quantification of the 5-HT$_{2A}$ receptor binding

The outcome parameter was the binding potential of specific tracer binding (BP_P). Tissue and plasma time activity levels were inspected to control for steady state. Cerebellum was used as a reference region since it represents non-specific binding only (Pinborg et al., 2003). In steady state, BP_P is defined as:

$$BP_P = \frac{C_{VOI} - C_{ND}}{C_P} = f_P \cdot \frac{B_{max}}{K_d}\ (ml/ml) \quad (1)$$

where C_{VOI} and C_{ND} are steady-state mean count density in the VOI and in the reference region, respectively, C_P is the steady-state activity of non-metabolized tracer 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.

Partial volume correction was performed according to Quarantelli et al. (2004). The white matter value was extracted as the mean voxel value from a predominantly white matter VOI (centrum semiovale) in the uncorrected PET image.

Voxel-based analysis

All subjects scanned with 3 Tesla MR ($n=69$) scan were included in a voxel-based analysis of parametric altanserin images. Univariate voxel-based analysis was conducted by means of statistical parameter mapping by employing the VBM5 toolbox (http://dbm.neuro.uni-jena.de) for spatial normalization/segmentation and SPM5 (Wellcome Trust Centre for Neuroimaging, London, United Kingdom) for modeling/statistical inference. The 5-HT_{2A} BP images were quantified according to the description in the section on Quantification of the 5-HT_{2A} receptor binding, and no partial volume correction was performed. All individual MR images were spatially normalized to the ICBM 152 (International Consortium for Brain Mapping) template in MNI space. Warp-fields were applied to each of the co-registered PET images, and these were subsequently resliced into MNI space with 2 mm isotropic voxels. After spatial normalization, PET images were smoothed with a 8 mm full width half maximum (FWHM) Gaussian kernel. Based on gray matter tissue maps a liberal mask for statistical analysis was defined by averaging over gray matter maps for all subjects and including voxels above a threshold of 0.3. Each regressor was zero meaned, while no further model scaling or normalization was performed. Statistical analysis was conducted by employing multiple regression with age, BMI and neuroticism as covariates. Significance levels for t statistics were set at $p<0.001$ (false discovery rate (FDR) corrected (Benjamini and Hochberg, 1995).

Statistics

The association between 5-HT_{2A} receptor binding and BMI was modeled using normal linear regression adjusting for MR type, age and neuroticism; the latter because an association between the personality trait and 5-HT_{2A} receptor binding has been demonstrated (Frokjaer et al., 2008). The non-partial volume corrected global neocortical region was chosen a priori as our primary outcome (dependent variable).

The main effects of gender, tobacco smoking and alcohol assumption and interactions between BMI and gender, and between BMI and the three allelic groups (G/G, A/G, and A/A) of the 5-HT_{2A} A(−1438)G promoter polymorphism were tested by including each term one by one in the model. Age, BMI and neuroticism were treated as continuous predictors, gender, type of MR scanner, tobacco smoking, and alcohol consumption as class variables. A multiple regression analysis including all terms was performed followed by backward elimination with cut-off at p-value of 0.05. Finally, all analyses were repeated using partial volume corrected data.

Variance homogeneity and normality were checked graphically. The linearity of quantitative variables was assessed by including second order terms in the models and thereby, model assumptions were found to be met.

In addition, the following analyses were performed:

1. To create a sample independent from our previously analyzed sample ($n=52$ Adams et al., 2004) and to avoid the potentially confounding effect of using two different MR scanners, an analysis was made on the subset of subjects scanned with the 3 Tesla MR-scanner only.
2. In the same subsample, regional differences were explored with a voxel-based analysis.
3. To rule out confounding effects of non-specific binding, the associations between non-specific binding and the following parameters were tested in the total sample of 136 subjects using a linear model with non-specific binding as dependent variable and adjustment for age: 1) BMI 2) the plasma fraction of metabolized radiotracer, and 3) C_P.
4. The associations between BMI and 1) plasma fraction of metabolized radiotracer, and 2) C_P were explored in a linear model with plasma fraction of metabolized radiotracer and C_P as dependent variable, respectively.
5. To examine if our primary observation was influenced by BMI-related differences in gray matter we tested the association between the gray matter ratio in neocortex and BMI in a linear model with adjustment for age, MR scanner type, and gender.
6. The effect of genotype was assessed. An Anova analysis of the association between BMI and the three allelic groups (G/G, A/G, and A/A) of the 5-HT_{2A} A(−1438)G promoter polymorphism were performed in a linear model with BMI as the dependent variable.
7. The association between familiar disposition to alcohol abuse and 5-HT_{2A} receptor binding was investigated in subsample of in 36 subjects (all scanned with 3 Tesla MRI) in a linear model with adjustment for age, neuroticism and BMI.

Group-comparisons between normal-weighted vs. overweight (BMI>25 kg/m^2) subjects were done for age, neuroticism, SCL, f_P, the plasma fraction of metabolized radiotracer, and injected cold altanserin dose, using unpaired t-tests.

A p-value<0.05 was considered statistically significant. p-values and parameter estimates with standard errors (SE) and 95% confidence limits are reported when appropriate.

Results

The overweight individuals (BMI>25 kg/m^2, $n=57$) did not differ from normal-weighted subjects (BMI≤25 kg/m^2, $n=79$) in f_P (0.040 ± 0.019 vs. 0.040 ± 0.017%, $p=0.88$), injected cold dose of altanserin (0.09 ± 0.07 vs. 0.09 ± 0.07 nmol/kg bodyweight, $p=0.99$), global SCL score (0.21 ± 0.22 vs. 0.19 ± 0.18, $p=0.57$), or neuroticism score (70 ± 17 vs. 72 ± 20, $p=0.72$). The overweight subjects were older (46.4 ± 18.6 vs. 35.8 ± 16.9 years, $p=0.001$) and had a higher plasma content of metabolized fraction of the radiotracer (48.4 ± 7.2 vs. 45.2 ± 7.7%, $p=0.015$) than the normal-weighted controls.

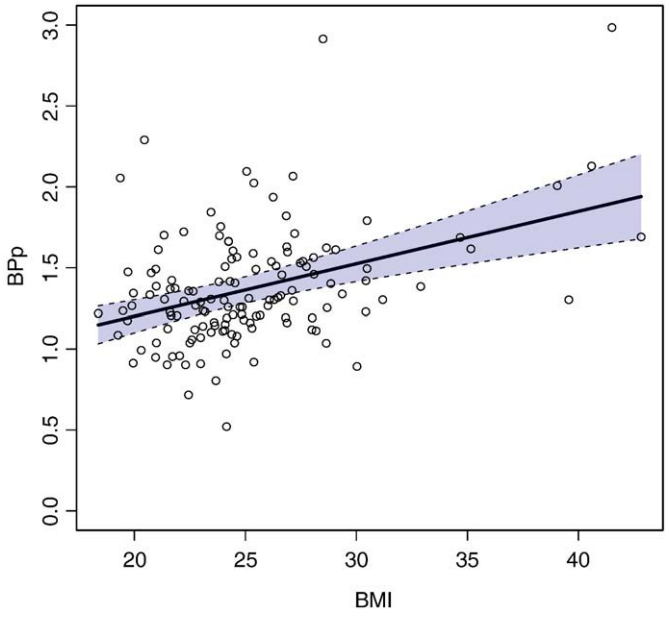

Fig. 1. Plot of BMI vs. neocortical 5-HT_{2A} receptor binding. The plotted BP_P values are the partial residuals from the linear model with BMI, age, neuroticism and MR-type (intercept defined at mean neuroticism, mean age and 1.5 Tesla MR-type).

Table 1
BMI effect on regional 5-HT_{2A} receptor binding in a multiple linear regression model adjusting for age, neuroticism, and MR type

Region	Estimate ± standard error (BP_P unit per kg/m^2)	95% confidence limits	*p*-value
Global neocortex	0.032 ± 0.007	(0.019–0.046)	<.0001
Anterior cingulate	0.039 ± 0.008	(0.023–0.055)	<.0001
Hippocampus	0.015 ± 0.004	(0.007–0.023)	.0003
Insula	0.035 ± 0.007	(0.020–0.049)	<.0001
Medial inferior frontal Ctx	0.033 ± 0.007	(0.019–0.047)	<.0001
Medial inferior temporal Ctx	0.035 ± 0.008	(0.020–0.050)	<.0001
Occipital Ctx	0.031 ± 0.007	(0.017–0.046)	<.0001
Orbitofrontal Ctx	0.043 ± 0.008	(0.028–0.058)	<.0001
Parietal Ctx	0.032 ± 0.007	(0.018–0.045)	<.0001
Posterior cingulate	0.031 ± 0.008	(0.016–0.046)	0.0001
Sensory motor Ctx	0.023 ± 0.005	(0.013–0.034)	<.0001
Superior frontal Ctx	0.030 ± 0.007	(0.017–0.043)	<.0001
Superior temporal Ctx	0.037 ± 0.008	(0.021–0.052)	<.0001

$n = 136$.

In the entire sample of 136 subjects, a significant positive correlation between BMI and 5-HT_{2A} receptor binding was found in all investigated brain volumes. The correlation was particularly pronounced in neocortex (0.032 BP_P per kg/m^2 (SD: 0.007, 95% confidence limits: 0.019 to 0.046), $p<0.0001$), please see Fig. 1. Data for the individual regions are presented in Table 1. Within the subsample of subjects scanned with 3 Tesla MRI ($n = 69$), the positive correlation between BMI and neocortical 5-HT_{2A} receptor binding was confirmed (0.037 BP_P per kg/m^2, $p<0.0001$), also in a voxel-based analysis (Fig. 2). The same findings were found for partial volume corrected data.

No significant association between gender and neocortical 5-HT_{2A} receptor binding with adjustment for BMI, age, and neuroticism was detected ($p = 0.72$ in the averaged neocortex). Neither was there any interaction between gender and BMI ($p = 0.155$). In all investigated brain regions, age was negatively correlated to BP_P (−0.017 BP_P per year (SD: 0.002, 95% confidence limits: −0.020 to −0.014), $p<0.0001$) whereas in all examined brain regions, the personality trait neuroticism correlated positively to BP_P (0.004 BP_P per unit neuroticism (SD: 0.002, 95% confidence limits: 0.001 to 0.007), $p = 0.0119$) in the averaged neocortex).

In the total sample of 136 subjects, the non-specific binding was 1.73 ± 4.7 (range: 0.63 to 3.65). A statistically significant positive correlation was found both between non-specific binding (cerebellar distribution volume) and BMI (0.024 per kg/m^2 (SD: 0.008, 95% confidence limits: 0.009 to 0.039), $p = 0.0020$), and between non-specific binding and the plasma fraction of metabolized radiotracer (0.029 per % unit (SD: 0.004, 95% confidence limits: 0.022 to 0.037), $p<0.0001$). There was also a positive correlation between the plasma fraction of metabolized radiotracer and BMI (0.394% unit per kg/m^2 per (SD: 0.148, 95% confidence limits: 0.101 to 0.686), $p = 0.0086$). There was no correlation between the gray matter ratio and BMI ($p = 0.1418$), or between C_{plasma} and BMI ($p = 0.5748$).

The 5-HT_{2A} A(−1438)G promoter polymorphism distribution was in Hardy–Weinberg equilibrium. No significant association was seen between neither the neocortical 5-HT_{2A} receptor binding and the A(−1438)G promoter polymorphism, nor between the 5-HT_{2A} receptor binding and the interaction between BMI and the A(−1438)G promoter polymorphism. Also, we found no differences in BMI within the 3 allelic groups (A/A, $n = 14$; A/G, $n = 44$; and G/G, $n = 37$) (24.4 ± 3.2, 25.0 ± 3.3, and 24.4 ± 3.3 kg/m^2 respectively). When A/A and A/G or A/G and G/G were pooled, there was still no between-group difference in BMI (24.9 ± 3.3 vs. 24.4 ± 3.3, and 24.7 ± 3.3 vs. 24.4 ± 3.2 kg/m^2).

In a model adjusting for age, neuroticism, BMI and MR scanner type, neither alcohol consumption (Fig. 3), nor tobacco use pattern (Fig. 4) was associated with 5-HT_{2A} receptor binding in any brain VOI (data other than averaged neocortex not shown). The lack of effect on 5-HT_{2A} receptor binding of being a smoker vs. non-smoker at present is not included in the figure.

Discussion

We found a positive correlation between BMI and in vivo cerebral 5-HT_{2A} receptor binding. We hereby replicate and extend the preliminary observation of a positive correlation between BMI and 5-HT_{2A} receptor binding in some brain regions (Adams et al., 2004), also in an independent sample. The relationship between BMI and global brain 5-HT_{2A} receptor binding was also confirmed in a voxel-based analysis.

Some possible sources of errors or confounds will be considered below. An obvious source of error is that a high BMI is associated with changes in radioligand metabolism or distribution in brain or plasma. After systemic injection, ^{18}F-altanserin gives yield to radiolabeled metabolites of which primarily radiolabeled altanserinol crosses the blood-brain barrier (Price et al., 2001a,b) and with a bolus-infusion protocol, the lipophilic metabolite(s) accumulate and increase the signal from non-specific binding over time (Pinborg et al., 2003). This

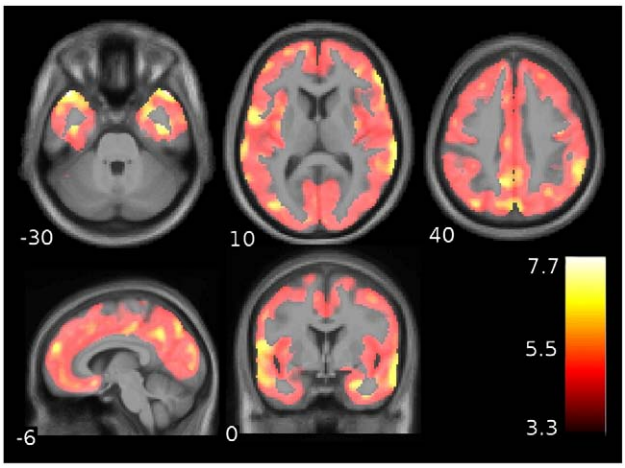

Fig. 2. Association between cerebral 5-HT_{2A} BP_P and BMI. Statistical parameter map (SPM) projected onto mean anatomical magnetic anatomical image of the 69 subjects. Age and neuroticism included as covariates in the general linear model (GLM). SPM thresholded to significance level $p<0.001$ (FDR corrected).

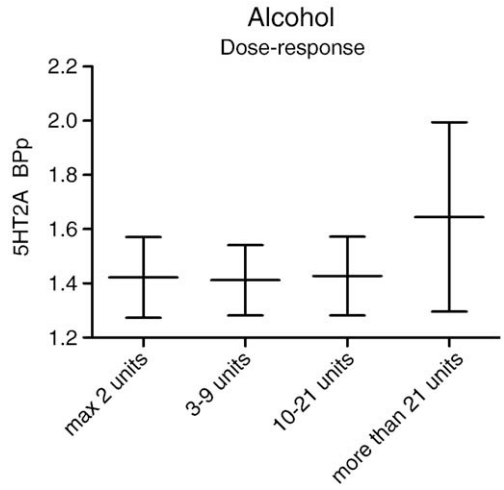

Fig. 3. Dose–response analysis of alcohol use vs. neocortical 5-HT_{2A} receptor binding. Subjects are divided into 4 groups based on the degree of their alcohol consumption (see Methods and materials section). BP_P values were adjusted for BMI, age, neuroticism and MR type. We found no significant difference in 5-HT_{2A} BP_P between the 4 groups (Anova $p = 0.6218$).

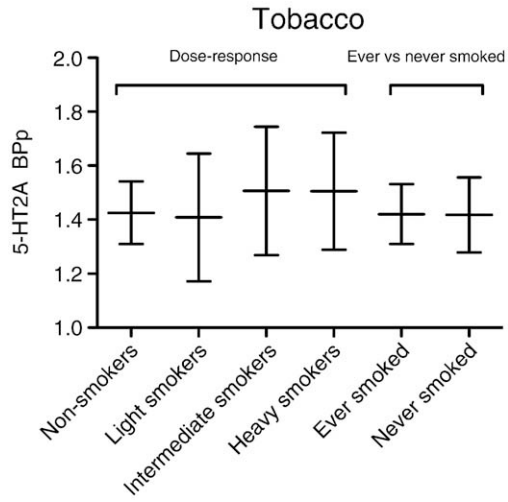

Fig. 4. Tobacco smoking vs. neocortical 5-HT_{2A} receptor binding. BP_P values were adjusted for BMI, age, neuroticism and MR type. Left: Dose–response analysis of tobacco smoking. Subjects are divided into 4 groups based on number of smoked cigarettes pr day (see Methods and materials section). Right: Comparison of BP_P between subjects who had smoked at present or earlier in life and subjects who had never been smoking. No significant difference in 5-HT_{2A} BP_P between the 4 groups or between the 2 groups (Anova $p = 0.7811$ and 0.9610, respectively).

notion was supported by our finding of a positive correlation between the metabolite fraction and non-specific binding, as measured in cerebellum. From Eq. (1), it can be seen that a relative underestimation of non-specific binding and/or C_P in overweight people would lead to an overestimation of the composite measure BP_P. We saw in our sample, by contrast, a *positive* correlation between BMI and non-specific (cerebellar) binding, which instead would tend to produce results in the opposite direction. This positive relationship was readily explained by the observation that a high BMI was associated with an increased plasma metabolite fraction. We also ruled out that there was any difference in the plasma free fraction of parent compound between normal-weighted and overweight subjects. Finally, no relationship was detected between BMI and C_P.

Most research on the serotonergic influence on appetite regulation has focused on 5-HT_{2C} and 5-HT_{1B} receptors; there is an abundant preclinical and clinical literature on the anorectic effects of serotonin 2C and 1B agonism (De Vry and Schreiber, 2000). If ^{18}F-altanserin was not selectively imaging the serotonin 2A receptor, but also the serotonin 2C receptors, the difference in ^{18}F-altanserin cortical receptor binding could potentially be caused by an upregulation of the serotonin 2C receptor. We consider this explanation unlikely since ^{18}F-altanserin has been shown to have a 5-HT_{2A} vs. 5-HT_{2C} receptor selectivity ratio of 20 (Tan et al., 1999), and in cerebral cortex the expression of 5-HT_{2A} is higher than 5-HT_{2C} receptors (Appel et al., 1990; Pompeiano et al., 1994; Nichols and Nichols, 2008). Finally, we have previously shown in brain homogenate binding studies that blocking with the 5-$HT_{2B/2C}$ selective compound SB 206553 does not alter ^{18}F-altanserin binding (Kristiansen et al., 2005).

In cerebral cortex, the 5-HT_{2A} receptor is predominantly located on pyramidal cells. Therefore, we explored if the correlation between BMI and 5-HT_{2A} receptor binding was driven by BMI-dependent differences in the amount of gray matter within the VOIs. In imaging studies using computerized tomography or MRI, others have found that overweight and obesity was associated with decreased gray matter volume (Gustafson et al., 2004; Pannacciulli et al., 2006; Taki et al., 2008). However, we did not identify such a relation between overweight and gray matter fraction.

Finally, one could speculate if the overweight and obese subjects had an overrepresentation of neuropsychiatric disorders, which could potentially contribute to the increase in 5-HT_{2A} binding. Absence of psychiatric disorders was, however, thoroughly assessed and in further support, neuroticism scores from the NEO-PI-R interview and symptom scores from the SCL interview did not differ between normal- and over-weighted subjects.

A recent study in mice supports our finding of an association between the cerebral 5-HT_{2A} receptor binding and BMI. Huang et al. demonstrated that mice that – when offered a high-fat diet – ate more and became obese displayed a higher cortical 5-HT_{2A} receptor density than their normal-weighted littermates on the same diet (Huang et al., 2004). This supports that an increased cerebral 5-HT_{2A} receptor binding is associated with increased food intake and body weight. The observed relation between 5-HT_{2A} receptor binding and BMI could either reflect a direct role of this receptor in regulation of appetite and food intake, or it could be secondary to other changes/dysregulations in the serotonergic neurotransmission. Support for a direct involvement comes primarily from studies of the 5-HT_{2A} receptor gene and body weight control. In a meta-analysis of nine genetic studies of anorexia nervosa, the A allele of the 5-HT_{2A} receptor G(−1438)A promoter polymorphism was shown to be associated with anorexia nervosa, although this vulnerability allele was suggested to be disorder modifying rather than causal (Gorwood et al., 2003). The positive relation between the G allele of the same polymorphism and body weight has also been established in studies of both adult overweight human subjects (Aubert et al., 2000; Sorli et al., 2008) as well as in normal weighted adults (Rosmond et al., 2002; Herbeth et al., 2005). In a group of 370 children and adolescents, Herbeth et al. did not see any relation between body weight and the G(−1438)A polymorphism but instead found that G allele carriers displayed a higher energy and fat intake than the A carriers (Herbeth et al., 2005). Together, data from these studies indicate that the expression of the 5-HT_{2A} gene could influence eating behavior in humans. However, it is possible that the association between the G(−1438)A polymorphism and food intake and/or body weight is more pronounced in subjects with a more extreme BMI, as suggested by Sorli et al. (2008). This, could also explain the discrepancies seen in studies relating the G(−1438)A promoter polymorphism to anorexia nervosa. We did not in our study observe any relation between the G(−1438)A promoter polymorphism and BMI and thus did not confirm the finding of higher BMI among G allele carriers of this polymorphism. Our sample size, however, was smaller than in the other studies and only a relatively small proportion of the subjects in our study were obese.

Currie et al. have in rats demonstrated that treatment with selective 5-HT_{2A} antagonists – in contrast to selective 5-HT_{2C} or 5-HT_{2B} antagonists – reverses the inhibitory effect of treatment with the 5-$HT_{2A/C}$ agonist DOI on neuropeptide Y induced hyperphagia (Currie et al., 2002). All these observations point to a specific role of 5-HT_{2A} receptor involvement in appetite regulation.

But there is also evidence to support the alternative view that our observation may be secondary to other changes in the serotonergic neurotransmission. Higher cerebral 5-HT_{2A} receptor binding in subjects with high BMI could also be secondary to lower cerebral 5-HT levels, caused by a dysfunctional regulation of the raphe innervation in these subjects. The observation of low cerebrospinal fluid levels of serotonin metabolites has been found in women with primarily abdominal obesity supports this notion (Bjorntorp, 1995). Interestingly, a negative relation between 5-HT_{2A} receptor binding and 5-HT levels is described in animal studies, with an increase in 2A binding after partial depletion (Heal et al., 1985; Cahir et al., 2007) and decreased binding after chronically increasing 5-HT levels with SSRI treatment (Cowen, 1990; Maj et al., 1996; Gunther et al., 2008). In disorders associated with dysregulation of the serotonin system, such as depression, the cerebral 5-HT_{2A} receptor binding is commonly interpreted as a marker of endogenous serotonin levels (Meyer, 2007). In line with this, it has been suggested that the decreased 5-HT_{2A} receptor binding observed in in-vivo imaging studies in patients

suffering from anorexia nervosa and bulimia nervosa patients subjects (Kaye et al., 2001; Audenaert et al., 2003; Bailer et al., 2004), is due to a compensatory downregulation in response to 5-HT hyperactivity (Frank et al., 2004; Kaye et al., 2005). The observation that both underweight patients with anorexia nervosa and recovered subjects have decreased 5-HT_{2A} receptor binding (Frank et al., 2002; Bailer et al., 2004), is suggestive of a primary serotonergic disturbance.

Future studies should in a longitudinal design explore whether weight loss or weight gain is associated with changes in the cerebral serotonin system. Likewise, it would be interesting to investigate the regulation of other serotonergic markers in relation to body weight, e.g., by exploring the relationship between cerebral serotonin transporter binding and BMI.

No relationship between 5-HT_{2A} receptor binding and the degree of alcohol consumption was detected in our study of non-alcoholic healthy subjects. The absence of severe drinkers may explain why we did not confirm the suggested implication of a dysregulated serotonergic neurotransmission in the pathophysiology of alcohol abuse (Heinz et al., 2004; Feinn et al., 2005). We used two different approaches to test if tobacco smoking among the subjects in our study was related to 5-HT_{2A} receptor binding, a dose–response analysis and "effect of ever smoked vs. never smoked" analysis. We did not detect any relationship using these two approaches between tobacco smoking and 5-HT_{2A} receptor binding.

Finally, we confirmed the age-dependent decline in cerebral 5-HT_{2A} receptor binding consistently reported before in both autoradiographical and in-vivo imaging studies (Arranz et al., 1993; Meltzer et al., 1998; Larisch et al., 2001; Meyer et al., 2003; Adams et al., 2004; Frokjaer et al., 2008). In this larger (overlapping) sample, we also confirmed the recently published observation by Frokjaer et al. that the personality trait neuroticism is positively correlated to 5-HT_{2A} receptor levels (Frokjaer et al., 2008). Gender did not affect 5-HT_{2A} receptor binding neither directly nor via an interaction with BMI.

Conclusion

We identified a positive correlation between cerebral cortex 5-HT_{2A} receptor binding and BMI. Whether the 5-HT_{2A} receptor has a direct role in the regulation of appetite and eating behavior or whether the finding is due to a compensatory upregulation of the receptor secondary to other dysfunction(s) in the serotonergic transmitter system, such as low baseline serotonin levels, remains to be resolved. We saw no association between past or current alcohol consumption or tobacco smoking and cerebral 5-HT_{2A} receptor binding.

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Study 2

"Cerebral serotonin transporter binding is inversely related to body mass index."

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Short title: "Cerebral SERT *vs.* BMI"

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PET; serotonin; serotonin transporter; imaging; obesity.

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Abstract

Serotonergic neurotransmission is critically involved in eating behavior. One piece of evidence for this is that the cerebral level of serotonin (5-HT) in animal models has been found inversely related to food intake and body weight. Extracellular brain levels of 5-HT are largely controlled by the presynaptically located serotonin transporter (SERT), and cerebral SERT levels do, on the other hand, adapt to chronic changes in extracellular 5-HT levels. We related regional cerebral SERT binding as measured with [^{11}C]DASB PET to body mass index (BMI) in 60 healthy volunteers. In a linear regression model with adjustment for relevant covariates, we found that cortical and subcortical SERT binding was negatively correlated to BMI (-0.003 to -0.012 BP_{ND} unit per kg/m^2). We speculate that SERT binding, in response to lower brain 5-HT levels, is lower in overweight individuals although the mechanism behind the relationship remains unclear.

Introduction

Overweight and obesity are conditions characterized by abnormal or excessive accumulation of body fat. Both conditions are associated with increased risks for developing diseases, such as type-2 diabetes, cardiovascular diseases, and certain types of cancer. For this reason, and because the frequency of overweight increases alarmingly world-wide the epidemic of overweight is today considered one of the most severe threats to human health. Globally, approximately 1.6 billion adults are overweight, and WHO has estimated that by 2015, 2.3 billion people will suffer from overweight (WHO 2006).

Body mass index (BMI), defined as the body weight divided by the squared height, is a commonly used measure for the nutrition state of an individual (Molarius and Seidell 1998). Individuals with a BMI of more than 25 kg/m^2 are designated overweight, whereas people with a BMI larger than 30 kg/m^2 are obese. Although the neurobiological mechanism behind overeating is only partly understood, an involvement of the serotonergic neurotransmission in eating behaviour and regulation of body weight has been suggested; Low brain levels of the monoamine serotonin (5-hydroxytryptamine, 5-HT) have been related to elevation of self-administration of food in animals (Breisch, Zemlan et al. 1976; Saller and Stricker 1976; Waldbillig, Bartness et al. 1981). Furthermore, 5-HT agonism has been related to weight loss in obese human subjects (Bever and Perry 1997) whereas depletion of 5-HT has been associated to an increase in food intake in women with bulimia nervosa (Weltzin, Fernstrom et al. 1995).

The evolutionarily highly conserved serotonin transporter (SERT) is a pre- and extrasynaptically localized membrane protein (Miner, Schroeter et al. 2000) that regulates the serotonin transmission via its reuptake of released 5-HT thereby modulating the extracellular fluid 5-HT concentrations. Drugs that increase the extracellular 5-HT through inhibition of SERT also inhibit food intake both in animals (Blundell 1984; Simansky 1996) and in humans (Olausson, Engel et al. 2002; Halford, Harrold et al. 2005; Johnson 2008). In addition, studies of SERT knockout mice have uncovered SERT as a candidate gene for human obesity, e.g., SERT mutant (SCL6A4-/-) mice become obese (Murphy and Lesch 2008) and in obese and overweight individuals, recent evidence points to a decreased expression of the gene

encoding for SERT (Sookoian, Gemma et al. 2007; Fuemmeler, Agurs-Collins et al. 2008).

In spite of these intriguing findings, only two in vivo molecular imaging studies of the SERT have been performed on overweight/obese subjects and both studies have employed non-selective monoamine transporter radioligands. In the first single photon emission tomography (SPECT) study, midbrain SERT binding was found to be lower in binge eating obese women than in non-binging obese women (Kuikka, Tammela et al. 2001). At re-examination after 8-24 months of SSRI treatment SERT binding in the binge eating obese subjects was increased (Tammela, Rissanen et al. 2003). In a recent [^{123}I]nor-β-CIT SPECT study including 16 monozygotic twin pairs, twins with a BMI higher than their monozygotic co-twins were found to have higher SERT binding (Koskela, Kaurijoki et al. 2008). In two independent large samples of healthy human subjects we previously detected a positive association between BMI and global neocortical 5-HT$_{2A}$ receptor binding (Adams, Pinborg et al. 2004; Erritzoe, Frokjaer et al. 2009) supporting the notion that overweight subjects might have lower cerebral 5-HT levels (Roth, Berry et al. 1998; Cahir, Ardis et al. 2007).

With the introduction of [^{11}C]DASB as a selective PET radioligand for SERT, reproducible quantification has become possible in multiple brain regions, even without arterial sampling (Houle, Ginovart et al. 2000; Ginovart, Wilson et al. 2001; Frankle, Slifstein et al. 2006; Kim, Ichise et al. 2006). In this study we investigated the cerebral SERT binding using [^{11}C]DASB PET in a large group human subjects representative of a Western population in terms of BMI. We hypothesized that we would find a negative correlation between BMI and SERT.

Methods and Materials

Participants and interviews

Sixty adult subjects (23 females, mean age 35.7 ± 18.2 years, and mean BMI 26.5 ± 5.9 kg/m^2) were included in the study. They were scanned between fall 2005 and spring 2008. Seven of the subjects had BMI above 30 and were thus classified as obese. Written informed consent was obtained according to the declaration of Helsinki II, and the study had been approved by the Copenhagen Region Ethics

Committee ((KF) 01-124/04, (KF) 01-156/04, and (KF) 01 2006-20, with amendments).

All subjects had a normal neurological examination and were lifetime naïve to antidepressants and antipsychotics. None of the subjects had stimulant abuse or history of neurological or psychiatric disorders. On the day of the PET scan, the subjects were screened for psychiatric symptoms using the Symptom Check List Revised (SCL-90-R) (Derogatis 1994). None of the subjects were depressed according to the cutt-offs from Danish normative data (Olsen, Mortensen et al. 2006). The Danish version of the 240-item NEO PI-R self-report personality questionnaire (Hansen 2004) was also filled out on the day of the PET scan. This questionnaire evaluates the personality dimensions of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness.

Subjects were interviewed about use of alcohol and tobacco smoking habits using an in-house made questionnaire. Based on their alcohol consumption, the subjects were divided into the following 3 categories: 1) subjects who drank maximum 2 units of alcohol per week (n=15); 2) subjects who drank 3 to 9 units of alcohol per week (n=24); 3) subjects who drank 10 or more units of alcohol per week (n=21). With regard to tobacco smoking, the subjects were divided into a group of tobacco smokers (n=12) and a group of non-smokers (n=48).

PET imaging

PET scans were performed with an 18-ring GE-Advance scanner (General Electric, Milwaukee, WI, USA), operating in 3D acquisition mode, and producing 35 images slices with an interslice distance of 4.25 mm. Following a 10 min transmission scan, a dynamic 90 minute long emission recording was initiated after intravenous injection over 12 sec of 475±92 MBq (range: 246 - 601) [^{11}C]DASB with a specific radioactivity of 30±16 GBq/μmol (range: 9 – 82). The emission recording consisted of 36 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 6 mm Hanning filter.

MR imaging

MR imaging of the brain was acquired on a Siemens Magnetom Trio 3T MR scanner with an eight-channel head coil (In vivo, FL, USA). High-resolution 3D T1-

weighted, sagittal, magnetization prepared rapid gradient echo (MPRAGE) scan of the head and 2D T2-weighted, axial, Turbo Spin Echo (TSE) scans of the whole brain were aquired. Both T1 and T2 images were corrected for spatial distorsions due to non-linearity in the gradient system if the scanner (Jovicich, Czanner et al. 2006) using the Gradient Non-Linearity Distorsion Correction software distributed by the Biomedical Informatics Research Network (hhtp://www.nbirn.net). Subsequently, non-uniformity correction was performed with two iterations of the N3 program (Sled, Zijdenbos et al. 1998). 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 using 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 (http://dbm.neuro.uni-jena.de/vbm/). This was done for the subcortical high-binding region and for neocortex, but not for midbrain because the segmentation within this region is not considered reliable. Therefore all midbrain voxels were included in the analysis. A brain mask based on the gradient non-linearity corrected T2 image was created in order to assure exclusion of extra-cerebral tissue.

Data analysis

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 (Woods, Cherry et al. 1992). The frames acquired for the first 2 minutes did not contain sufficient 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 the thresholded images to ensure that they included the bran gray matter voxels. The rigid transformation was estimated for each frame to a selected single frame with enough structural information (frame 26: 20-25 minutes 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 image (based on an average of frame 10 – 36) was co-registered to the individual MR image using the AIR algorithm (Woods, Cherry et al. 1992). The quality of each co-registration was assessed visually in three planes and adjusted when needed (this was needed in 3 cases).

VOI analysis

Volumes of interest (VOIs) were automatically delineated on each subject's MR image in a user-independent fashion with the Pvelab software package (freely available on www.nru.dk/downloads) (Svarer, Madsen et al. 2005). For each of the 10-template VOI sets, a 12-parameter affine transformation and a warping field were calculated between the template MR image and the individual MR image for a subject. Having obtained the MR/PET co-registration for the same individual as described above, the template VOI sets are then transferred to the dynamic PET image space for each subject, using the identified transformation parameters. From the VOI sets, a probability map was created for each subject and a common VOI set was generated for each individual subject. These VOI sets were then used for automatically extraction of time activity curves (TAC) for each of the VOIs. The TAC extracted for the cerebellum, excluding the cerebellar vermis (Kish, Furukawa et al. 2005), was used as the reference tissue input for kinetic modeling.

As a robust measure of cerebral SERT binding, a high-binding subcortical region consisting of 3 paired regions with high, homogenous binding (Houle, Ginovart et al. 2000) was computed as the volume-weighted average of binding in caudate, putamen and thalamus. Together with the midbrain region and a neocortical region, this high-binding region served as the primary VOI. The neocortical region consisted of a volume-weighted average of the following 8 cortical regions: Orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, superior temporal cortex, medial inferior temporal cortex, sensory motor cortex, parietal cortex, and occipital cortex. The deliniation of all VOIs except the midbrain have been described previously (Svarer, Madsen et al. 2005). Midbrain was defined in the ACPC plane (the plane of the anterior and posterior commissure) as the superior limit and the boarder between the inferior colliculi and the superior cerebellar peduncle as the inferior limit. In the 2-3 most superior slices where the peduncle is less well defined, only tegmentum and tectum were included in the region. Within the high-

binding subcortical region and neocortex, the ratio between gray matter volume and the sum of the white plus gray matter volumes was computed.

Quantification of non-displaceable tracer uptake.

The outcome parameter from the [^{11}C]DASB-PET study is the non-displaceable binding potential, designated BP_{ND}(Innis et al, 2007). Cerebellum (as defined above) was used as a reference region, representing non-specific binding only. We used a modified reference tissue model designed specifically for quantification of [^{11}C]DASB (MRTM/MRTM2) as described and evaluated by Ichise et al. (Ichise, Liow et al. 2003) using the software PMOD version 2.9, build 2 (PMOD Technologies): A fixed washout constant, designated k2', was calculated for each individual as an average of k2 in caudate, putamen and thalamus relative to cerebellum using MRTM. Subsequently, k2' was inserted into MRTM2 and BP_{ND} was calculated for the VOIs relative to cerebellum.

Statistics

The association between BMI and SERT binding in the three VOIs was analyzed in a linear regression model with adjustment for age, gender, minutes of daylight on the scan date at the latitude of Copenhagen (http://aa.usno.navy.mil/data/docs/Dur_OneYear.php/), and openness to experience. Daylight was included in this full model because of it's association with SERT binding (Praschak-Rieder, Willeit et al. 2008), and opennes to experience was included because of relation to SERT binding shown in 50 subjects overlapping the cohort in the present study (Kalbitzer, Frokjaer et al. 2009). In a subsequent analysis, model simplification was performed by backward elimination with cut-off at a p-value of 0.05. To examine if our primary observation was influenced by BMI-related differences in gray matter, we tested the linear association between the cortical and subcortical gray matter ratio and BMI. Variance homogeneity and normality were validated graphically. The linearity of quantitative variables was assessed by including second order terms in the models which in all cases were statistical non-significant. Finally, in a post-hoc analysis the association between SERT binding and tobacco smoking and alcohol consumption was evaluated in both the full and in the reduced model.

Group-comparisons between normal-weighted and overweight subjects were performed with a t-test. Comparisons included age, injected mass of cold ligand, specific activity of [^{11}C]DASB, openness to experience, daylight minutes, the area under the cerebellar time-activity curves normalized to the injected dose, and the reference tissue wash-out k2´.

A p-value<0.05 was considered statistically significant. P-values, parameter estimates with standard errors (SE) and 95% confidence limits, and degrees of freedom (DF) are reported when appropriate. We used SAS software (SAS Institute Inc.) version 9.1.3 for statistical analysis.

Results

Figure 1 shows the averaged parametric BP_{ND} image of the SERT binding. In the full linear model where the BMI effect on SERT binding was adjusted for age, gender daylight minutes, and openness to experience, BMI correlated statistically significant negatively to SERT binding in neocortex (-0.003 BP_{ND} unit per kg/m^2 (SE: 0.001, 95% confidence limits: -0.005 to -0.001), p=0.017) and in the subcortical high-binding region (-0.009 BP_{ND} unit per kg/m^2 (SE: 0.004, 95% confidence limits: -0.018 to -0.0003), p=0.042). In midbrain, the inverse relationship was borderline significant only (-0.010 BP_{ND} unit per kg/m^2 (SE: 0.005, 95% confidence limits: -0.021 to 0.001), p=0.066).

The overweight individuals (BMI>25, n=36) did not differ from normal-weighted subjects (BMI≤25, n=24), with regard to injected mass (69.9±34.7 vs. 79.5±40.7 ng per kg body weight, p=0.349), [^{11}C]DASB specific activity (29±15 vs. 31±17 GBq/μmol, p=0.558), openness score (113±19 vs. 120±18, p=0.184), number of daylight minutes on the day of the individual PET scan (700±236 vs. 657±233, p=0.486), area under the cerebellar time-activity curves normalized to the injected dose (136145±29535 vs. 146157±32961, p=0.236), or in the reference tissue wash-out k2´ (0.054±0.009 vs. 0.056±0.008, p=0.433). There was a tendency for overweight subjects to be slightly older than the normal-weighted controls (38.9±19.0 vs. 31.0±16.2 years, p=0.090).

After backward elimination of parameters that did not contribute significantly to the full model description (see above), an even stronger inverse correlation between BMI and SERT binding was found in all three VOIs. The regional data from this

regression analysis is presented in table1, and high-binding subcortical SERT BP_{ND} is plotted against BMI in figure 2.

For the subcortical high-binding region, there were no statistically significant interactions between age and BMI or between daylight minutes and BMI. In midbrain we found a main effect of gender on SERT binding, with females having a higher SERT binding. There were no significant interactions between BMI and openness, or between BMI and age, or BMI and gender. For neocortex, there was no significant interaction between age and BMI. BMI was categorized into 3 categories when looking for interactions with another continuous variable. No significant correlation was detected between BMI and grey matter ratio in any VOI.

No effect of tobacco smoking or alcohol consumption on SERT binding was found in any of the three VOIs. This was true both for the full model with inclusion of all co-variates, and for the reduced model.

Discussion

In this relatively large sample of 60 healthy people, representative of a Western population in terms of BMI, we found a negative correlation between BMI and in vivo SERT binding in all three investigated brain regions, ranging from high to low binding: midbrain, caudate-putamen-thalamus, and neocortex. Potential confounds such as differences between overweight/obese and normal weighted subjects with regard to non-specific binding and injected mass, should be considered. The quantification with a tissue reference model without arterial sampling excludes a proper assessment of the individual cerebellar non-specific binding. In theory, a false negative correlation could occur if subjects with high BMI had higher non-specific binding than those with low BMI. However, as a proxy for non-specific binding, neither the area under the cerebellar time-activity curves normalized to the injected dose nor reference tissue wash-out, k2, differed between groups indicating that differences in non-specific binding did not explain group differences in specific SERT binding. A group difference in injected mass could potentially also influence the outcome; if more cold mass was administered to the overweight/obese subjects a larger fraction of the transporters could be blocked yielding a false low BP_{ND} within these subjects. The injected mass did not differ between the groups and thus could not explain the finding. Further, The injected mass in our study was in average 73.7±37.2 ng per kg body weight corresponding to a transporter occupancy smaller than 0.5%

(Ginovart, Wilson et al. 2003). We expected a non linear association between age and bmi and hence the inclusion of both predictors in a linear model should not cause severe problems with collinearity. This was confirmed by examination of variance inflation factors as well ridge regression estimates.

Also, there were no group-differences between openness to experience or number of daylight minutes on the day of the PET scan and none of these parameters interacted with BMI in the linear model.

We suggest that the negative correlation between BMI and "global" in vivo cerebral SERT binding is mediated through interindividual variations in cerebral 5-HT levels that are reflected in SERT levels. In animal models, manipulations that decrease brain 5-HT neurotransmission lead to elevated self-administration of food whereas treatments that increase 5-HT levels induce satiety which subsequently lead to decreased food intake and weight loss (Saller and Stricker 1976; Waldbillig, Bartness et al. 1981)((Blundell 1984; Simansky 1996; Leibowitz and Alexander 1998). The observation of low cerebrospinal fluid levels of serotonin metabolites in women with primarily abdominal obesity (Strombom, Krotkiewski et al. 1996) and the demonstration of increased food intake in women suffering from bulimia nervosa after lowering brain 5-HT levels by tryptophan depletion support that also in humans, low cerebral synaptic 5-HT levels increases appetite, food intake and subsequently lead to overweight (Weltzin, Fernstrom et al. 1995; Smith, Fairburn et al. 1999). In two independent large samples of healthy human subjects we previously detected a positive association between BMI and global neocortical 5-HT_{2A} receptor binding (Adams, Pinborg et al. 2004; Erritzoe, Frokjaer et al. 2009) in support of the notion that overweight subjects might have lower cerebral 5-HT levels (Roth, Berry et al. 1998; Cahir, Ardis et al. 2007).

Since 5-HT levels cannot readily be determined in vivo in the human brain there are no clinical data available on the association between cerebral 5-HT levels and SERT binding. There is, however, evidence from animal studies to support that changes in 5-HT levels lead to an inverted U-shaped regulation of SERT-binding; chronic 5-HT depletion in animals leads to downregulation of SERT binding (Rattray, Baldessari et al. 1996; Rothman, Jayanthi et al. 2003) and a change in binding in the same direction is observed after increasing 5-HT levels with SSRI treatment (Pineyro, Blier et al. 1994; Benmansour, Cecchi et al. 1999; Horschitz, Hummerich et al. 2001; Benmansour, Owens et al. 2002; Gould, Pardon et al. 2003; Gould, Altamirano et al.

2006). Acute tryptophan depletion in humans, where a rapidly reversible reduction occurs in cerebral 5-HT levels, does not alter cerebral [^{11}C]DASB binding (Praschak-Rieder, Wilson et al. 2005; Talbot, Frankle et al. 2005) in accordance with the notion that transporter internalization and subsequent degradation is a more long-term process, perhaps in the order of 14 days (Rattray, Baldessari et al. 1996). In both animals and human, the administration or use of MDMA (methylene-dioxy-methamphetamine or ecstasy) is associated with decreased cerebral SERT binding (Cowan 2007). MDMA is a potent 5-HT releaser in the acute phase, but is followed by a more chronic depletion of 5-HT (Morton 2005). Although MDMA has its limitations as a pure 5-HT depletion model, it is consistently reported that SERT binding is low in chronic MDMA users (Reneman, Endert et al. 2002; McCann, Szabo et al. 2005; Buchert, Thiele et al. 2007). Interestingly, mice exposed to MDMA show a biphasic feeding response with hypophagia within the first hour followed by hyperphagia, in agreement with the expected effects of an intially high, then reduced cerebral 5-HT levels (Conductier, Crosson et al. 2005).

The feeding pattern associated with a 5-HT reduction is altered towards both intake of smaller meals, slower eating but unchanged meal frequency (Blundell 1984; Leibowitz 1988; Simansky 1996), suggesting that 5-HT modulates satiety. It has been shown that serotonergic acting agents, especially when injected directly into the hypothalamus, suppress carbohydrate consumption while having little or no effect on the ingestion of protein or fat (Leibowitz and Alexander 1998). Likewise, carbohydrate ingestion leads to increased circulating levels of the 5-HT amino acid precursor, tryptophan (Fernstrom, Faller et al. 1975; Noach 1994; Wurtman and Wurtman 1995), as well as increased hypothalamic and raphe nuclei 5-HT (Leibowitz and Alexander 1998). Thus, 5-HT as a feedback on eating, serves to terminate the meal and yield a state of satiety. As obese subjects tend to have a preference for carbohydrate rich food (Weltzin, Fernstrom et al. 1994; Wurtman and Wurtman 1995) it is possible that these subjects have a disturbed 5-HT mediated feedback. From these observations, the physiology of normal eating behaviour seem to be related to serotonergic transmission and a serotonergic dysfunction seem to play an important part in the brain pathophysiology behind eating disorders through a disturbance of these satiety mechanisms eventually leading to overeating and obesity. In line with this, our observations of a negative association between BMI and SERT binding concurs well with the previously observed positive association to 5-HT$_{2A}$ receptor

binding, in that both associations could be jointly explained by lower brain 5-HT levels in individuals with high BMIs.

A negative correlation between BMI and cerebral SERT binding has also been identified in a preliminary report by Matsoumoto et al (Matsumoto 2008) based on 25 healthy human subjects. In addition, a relationship between impaired serotonergic transmission and binge eating behavior has been suggested by Kuikka et al who detected reduced midbrain SERT binding in binge eating obese women (Kuikka, Tammela et al. 2001). In contrast, twins with higher BMI were found to have higher SERT binding, as measured with [^{123}I]nor-β-CIT SPECT, than their monozygotic twin sibling with lower BMI (Koskela, Kaurijoki et al. 2008). In the latter study, BMI did not correlate with SERT binding in the entire group of 31 subjects (one subjects excluded from this analysis). The reason for the discrepancy between our and Matsumouto et al's results on one hand and Koskela et al's on the other, is not readily explained but the authors of the latter study (Koskela, Kaurijoki et al. 2008) suggest that [^{123}I]nor-β-CIT in contrast to other more thoroughly validated SERT ligands might be sensible to endogenous 5-HT levels. Although this issue has never been settled, then a reduced 5-HT would result in a higher SERT binding, as measured with [^{123}I]nor-β-CIT-SPECT.

As an alternative to the suggested explanation for our finding, the inverse relationship between BMI and cerebral SERT binding could also be due to shared genetic and/or early environmental factors. SERT knockout mice, when aged approximately 3 months, become obese (Holmes, Murphy et al. 2002; Warden, Robling et al. 2005; Murphy and Lesch 2008). In further support of a genetic component in the involvement of serotonergic neurotransmission in regulation of body weight, are two recent publications in which an association between overweight/obesity and the s allele of the SLC6A4 HTTLPR polymorphism has been demonstrated in Argentinean adolescents (Sookoian, Gemma et al. 2007) and in Hispanics and American white men (Fuemmeler, Agurs-Collins et al. 2008). Since evidence suggests that the s-allele confers decreased SERT expression and binding sites (Lesch, Bengel et al. 1996; Little, McLaughlin et al. 1998; Heinz, Jones et al. 2000), especially when taking the triallelic variation in the 5-HTTLPR into account (Praschak-Rieder, Kennedy et al. 2007; Reimold, Smolka et al. 2007) this is in good agreement with our finding. However, not all data support a such relation between 5-

HTTLPR polymorphism and SERT density (Greenberg, Tolliver et al. 1999; Mann, Huang et al. 2000; Preuss, Soyka et al. 2000; van Dyck, Malison et al. 2004; Parsey, Hastings et al. 2006).

Adaptive changes of the serotonergic neurotransmission to environmental factors during early development could also take place. For example, protein restriction during early development leads to an attenuation of the inhibitory action of 5-HT on food intake (Lopes de Souza, Orozco-Solis et al. 2008). Finally, other interpretations such as decreased density of SERT expressing neurons or dendrites with increased BMI, should be considered. In imaging studies using computerized tomography or MRI, others have found that overweight and obesity was associated with decreased gray matter volume in various brain regions (Gustafson, Lissner et al. 2004; Pannacciulli, Del Parigi et al. 2006; Taki, Kinomura et al. 2008). In our sample, we were not able to identify any relation between BMI and the fraction of gray matter in any region.

In conclusion, it remains unclear whether the findings of relations between binding potentials of 5-HT markers and BMI represent adaptive changes, degenerative etiological mechanisms, or if they should be considered as an unrelated phenomenon.

No relationship between SERT binding and the degree of alcohol consumption was detected in our study of these non-alcoholic healthy subjects. The absence of severe drinkers may explain why we did not confirm the suggested dysregulation of 5-HT neurotransmission in alcohol abusers (Heinz, Goldman et al. 2004; Feinn, Nellissery et al. 2005). Further, we did not detect any effect of smoking on SERT binding. It should be noted that our study was not directly designed to examine the effect of smoking and therefore relatively few tobacco smokers (n=12) were included in the study. Accordingly, we cannot exclude that an effect of smoking could be demonstrated in a study with sufficient power to study this specific question.

We found that in all investigated brain regions, SERT-binding decreased with age. This supports that age needs to be considered in SERT studies, in line with some (Meyer, Wilson et al. 2001; Reimold, Smolka et al. 2007) but not all imaging studies (Meyer, Houle et al. 2004). A significant effect of gender was only observed in midbrain where females displayed the highest SERT binding. This is supported by one study using the non-selective SPECT tracer [^{123}I]-beta-CIT (Staley, Krishnan-Sarin et al. 2001). Yet other studies using selective tracers have not confirmed a

gender difference (Meyer, Houle et al. 2004) or have observed lower SERT binding in females in both cortical and subcortical regions (Jovanovic, Lundberg et al. 2008). Thus, the gender influence on SERT availability is not fully clear.

Our study has some limitations. First, when reporting SERT binding from cortical areas with [^{11}C]DASB PET it should be emphasized that because of the relatively low SERT binding in these areas the interindividual variability is high and the signal to noise ratio is low. Consequently, evaluating data from cortical brain regions should be with caution. However, in a test-retest study using the same method as used in our study ([^{11}C]DASB-PET and MRTM2), except for longer scan time, a high cortical reliability was shown (temporal 0.82, occipital 0.85, and frontal 0.55)(Kim, Ichise et al. 2006). In addition, the detected relation seems to be a global cerebral phenomenon and since the regional BP_{nd} values only to a certain extent intercorrelate (data not shown), it seems reasonable to believe that the relation encompasses the cortex. Secondly, the binding potential was included as the dependent variable in the statistical analysis. In the present study, as well as in our prior analysis of 5-HT_{2A} receptor binding (Adams, Pinborg et al. 2004) we chose the same approach. One could argue, however, that the causality between BMI and serotonergic neurotransmisson should be reverted and that a more meaningful model would therefore include BMI as the dependent variable. When we analyzed our data with a linear regression model with adjustment for age and gender and with BMI as explained by SERT binding, we confirmed a statistically significant negative association in neocortex and the averaged caudate-putamen-thalamus region, and a trend for a negative association in midbrain. However, with this approach, the potential feedback mechanism from changes in eating behavior and body weight on the 5-HT neurotransmission is disregarded. To further address the causality, exploration in a longitudinal set-up with intervention would be needed; e.g., to study the effect on brain serotonergic markers in response to a substantial weight loss.

Conclusion

Cerebral SERT binding is inversely correlated to BMI. Whether the serotonin transporter has a direct role in the regulation of appetite and eating behavior or whether the finding is due to a compensatory downregulation of the transporter secondary to other dysfunction(s) in the serotonergic transmitter system, such as low baseline serotonin levels, remains uncertain.

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Disclosure/Conflict of Interest

The authors declare that, except for income received from primary employers, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

Figures and Tables

Fig 1

Parametric image of averaged BP_{ND} values for all 60 subjects. Average BPnd values: thalamus 1.7; caudatus 1.4; putamen 1.7; midbrain 1.8 , neocortex 0.2.

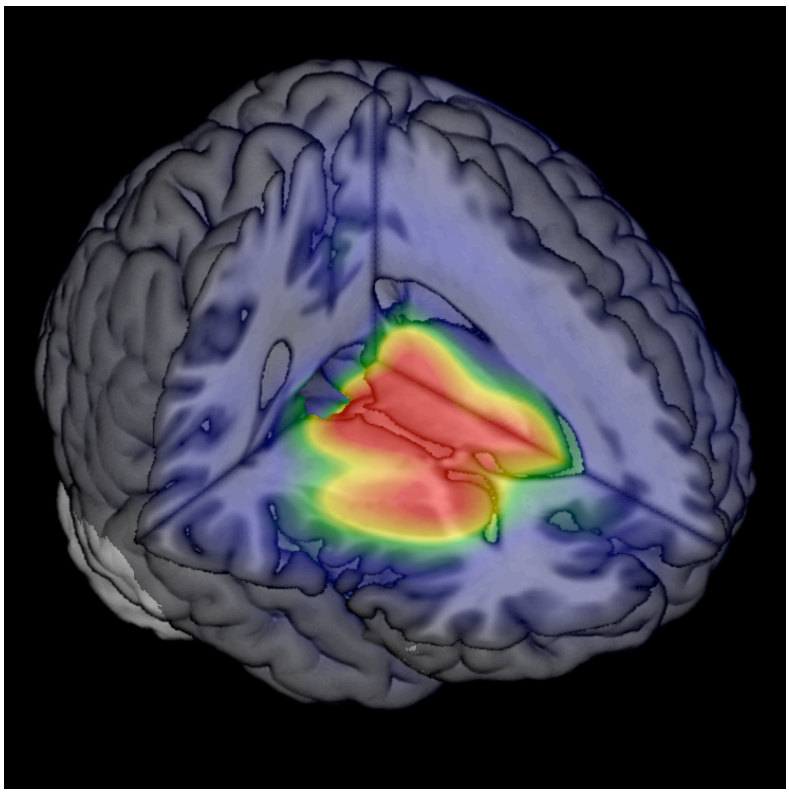

Fig 2

Plot of BMI vs. subcortical high-binding (caudatus-putamen-thalamus) SERT binding. The plotted BP_{ND} values are the partial residuals with 95% pointwise confidence limits from the linear model with BMI, age (centered), and daylight minutes as co-variates.

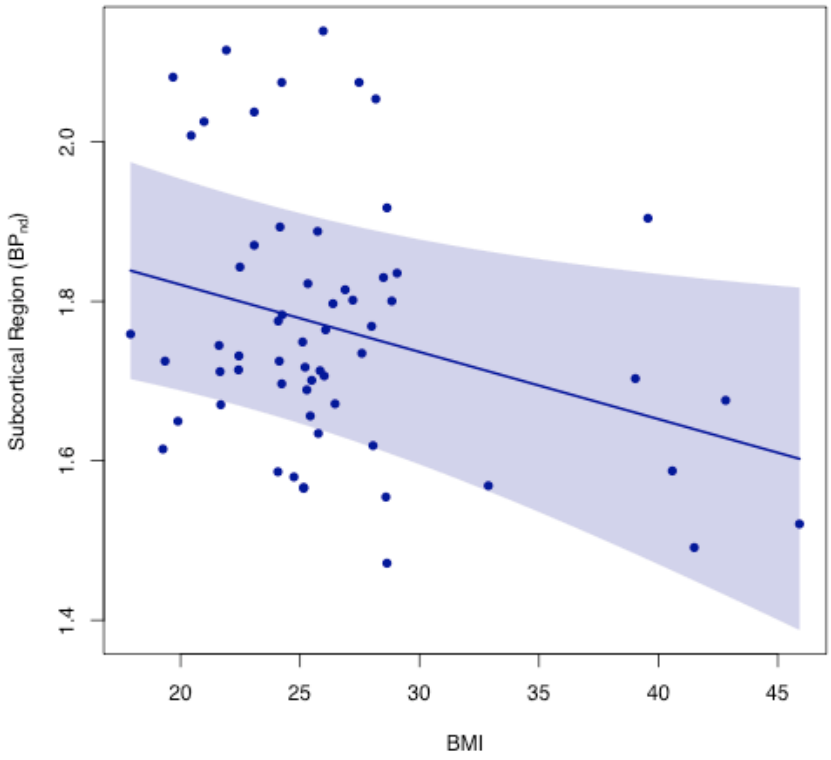

Table 1

The main effect on regional SERT binding of significant parameters after backward-elimination in a multiple linear regression model.

Region	Estimate ± Standard Error	95% Confidence Limits	P-value
Caud-Put-Thal			
BMI	-0.008 ± 0.004 (BP_{nd} unit per kg/m^2)	-0.016 to -0.001	p=0.027
Age	-0.003 ± 0.001 (BP_{nd} unit per year)	-0.006 to -0.001	p=0.006
Daylight minutes	-0.0002 ± 0.00009 (BP_{nd} unit per min)	-0.0004 to -0.000001	p=0.048
Midbrain			
BMI	-0.012 ± 0.005 (BP_{nd} unit per kg/m^2)	-0.022 to -0.001	p=0.027
Age	-0.004 ± 0.001 (BP_{nd} unit per year)	-0.007 to -0.001	p=0.019
Openness	-0.004 ± 0.002 (BP_{nd} unit per Op. unit)	-0.007 to -0.001	p=0.014
Gender	-0.176± 0.059 (BP_{nd} unit; ref: female)	-0.294 to -0.057	p=0.004
Global Neocortex			
BMI	-0.003 ± 0.001 (BP_{nd} unit per kg/m^2)	-0.005 to -0.001	p=0.003
Age	-0.001 ± 0.0003 (BP_{nd} unit per year)	-0.002 to -0.001	p<0.001

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Study 3

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Cortical and Subcortical 5-HT_{2A} Receptor Binding in Neuroleptic-Naive First-Episode Schizophrenic Patients

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The serotonin 5-HT_{2A} receptor is suspected to be involved in a number of psychiatric disorders, including schizophrenia. In particular, atypical antipsychotics have antagonistic effects on the 5-HT_{2A} receptors, supporting a specific role of the 5-HT_{2A} receptor in the pathophysiology of this disease. The aim of this study is to investigate cortical and subcortical 5-HT_{2A} binding in neuroleptic-naive schizophrenic patients. Fifteen neuroleptic-naive patients diagnosed with schizophrenia (age 27.5 ± 4.5 years), 11 men and 4 women, and 15 healthy control subjects matched for age (28.5 ± 5.7 years) and gender underwent a 40 min positron emission tomography (PET) study using the 5-HT_{2A} antagonist, [^{18}F]altanserin, as a radioligand. PET images were co-registered to 3 T magnetic resonance images (MRIs) for each individual subject, and ROIs were applied automatically onto the individual MRIs and PET images. The cerebellum was used as a reference region. The binding potential of specific tracer binding (BP_p) was used as the outcome measure. No significant difference was seen in cortical receptor distribution between patients and controls. An increase in 5-HT_{2A} receptor binding in the caudate nucleus was detected in the group of schizophrenic patients (0.7 ± 0.1) when compared to the healthy controls (0.5 ± 0.3) ($p = 0.02$). Our results confirm other *in vivo* findings of no difference in cortical 5-HT_{2A} receptor binding between first-episode antipsychotic-naive schizophrenic patients and age- and gender-matched healthy control subjects. However, a preliminary finding of increased 5-HT_{2A} binding in the caudate nucleus requires further investigation to explore the relation of subcortical and cortical 5-HT_{2A} receptor binding.

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INTRODUCTION

A role for serotonin in the pathophysiology of schizophrenia is supported by different observations. Gaddum (1954) described that the hallucinogenic drug lysergic acid diethylamide had structural similarity to serotonin and could cause or exacerbate psychotic symptoms. These early findings led to the hypothesis that serotonin was implicated in the pathophysiology of schizophrenia. Further support for this comes from the notion that most atypical antipsychotic drugs (AAPDs) antagonize the serotonin 2A (5-HT_{2A}) receptor. The affinity of AAPDs as determined *in vitro* (Meltzer *et al*, 1989) and *in vivo* (Zhang and Bymaster, 1999) is often higher for 5-HT_{2A} than D_2 receptors. Finally, post-mortem studies suggest a serotonergic dysfunction in cortical areas in schizophrenia. Eleven (Arora and Meltzer, 1991; Bennett, 1979; Burnet *et al*, 1996; Dean and Hayes, 1996; Dean *et al*, 1998, 1999; Gurevich and Joyce, 1997; Laruelle *et al*, 1993; Matsumoto *et al*, 2005; Mita *et al*, 1986; Pralong *et al*, 2000) out of 15 (Dean *et al*, 1996; Joyce *et al*, 1993; Reynolds *et al*, 1983; Whitaker *et al*, 1981) post-mortem studies of brains of schizophrenic patients have reported decreased 5-$HT_{2A/C}$ density in cortical areas, especially in the frontal cortex. Only two studies have addressed the subcortical 5-HT_{2A} density in post-mortem material: Joyce *et al* (1993) found an increased 5-HT_{2A} density in the ventral putamen and nucleus accumbens, whereas Matsumoto *et al* (2005) reported no significant difference in striatum between controls and patients suffering from schizophrenia.

Investigations with *in vivo* imaging techniques have not supported post-mortem findings of a cortical decrease in

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5-HT$_{2A}$ receptor binding in schizophrenia. Three positron emission tomography (PET) studies showed no difference in 5-HT$_{2A/C}$ receptor density between schizophrenic patients and controls (Lewis *et al*, 1999; Okubo *et al*, 2000; Trichard *et al*, 1998). Only one study revealed a decreased 5-HT$_{2A}$ binding potential in the frontal cortex of six neuroleptic-naive schizophrenic subjects when compared to healthy controls (Ngan *et al*, 2000). The latter publication was based on a voxel-based image analysis, whereas the three prior ones were based on a region-based analysis. All four previous studies were performed on a limited number of patients and only some of them were antipsychotic-naive. Three of the studies used [^{18}F]setoperone as a 5-HT$_{2A}$ tracer, whereas one study used [^{11}C] *N*-methylspiperone. Owing to a relatively poor selectivity of both radiotracers for the 5-HT$_{2A}$ receptor, these studies are limited to the detection of receptor binding only in cortical areas. In conjunction with the poor selectivity of the tracers, a lower ratio of 5-HT$_{2A}$ receptors to D$_2$ receptors in subcortical areas compared to cortical areas makes these ligands inadequate to measure subcortical binding. Today, two other specific 5-HT$_{2A}$ radioligands are available: [^{11}C]MDL 100,907 and [^{18}F]altanserin. [^{18}F]altanserin has a 200- to 500-fold 5-HT$_{2A}$/D$_2$ selectivity measured as 1/(5-HT$_{2A}$ K_i/D$_2$ K_i) = 1/(0.13–0.3/62 nM) = 1/(0.002–0.005) (Kristiansen *et al*, 2005; Tan *et al*, 1999), making it between 8 and 50 times more selective for the 5-HT$_{2A}$ receptors than [^{18}F]setoperone ((1/(1/10–25 nM) = 1/(0.1–0.04) = 10–25-fold 5-HT$_{2A}$/D$_2$ selectivity (Lewis *et al*, 1999)). In addition, the affinity of [^{18}F]altanserin for the 5-HT$_{2A}$ receptor is at least 20-fold higher than for other 5-HT subtypes (Tan *et al*, 1999). We have previously demonstrated that [^{18}F]altanserin PET with a bolus infusion design is a highly reproducible method for reliable quantification of 5-HT$_{2A}$ receptor binding (Haugbøl *et al*, 2007).

The aim of the present PET study was to investigate cortical and subcortical 5-HT$_{2A}$ receptor binding in a group of first-episode antipsychotic-naive schizophrenic patients and matched healthy controls using [^{18}F]altanserin PET.

MATERIALS AND METHODS

Participants

Fifteen patients (11 men and 4 women) were recruited after voluntary first-time referral to a psychiatric unit of one of the affiliated university hospitals in the Copenhagen area (Bisbebjerg Hospital, Rigshospitalet, Psychiatric University Centre Glostrup or Psychiatric University Centre Gentofte). The study was approved by the Ethics Committee of Copenhagen and Frederiksberg ((KF)11-061/03). The subjects participated after receiving a full explanation of the study and providing written informed consent according to the declaration of Helsinki II.

The patients included fulfilled diagnostic criteria for schizophrenia according to both ICD-10 and DSM IV. All patients were antipsychotic-naive at the time of investigation. Diagnosis was verified by means of the structured clinical interview SCAN 2.1 (Schedules for Clinical Assessment in Neuropsychiatry). The severity of symptoms in subjects was assessed with Positive and Negative Syndrome Scale (PANSS). All interviews were recorded on video for validation purposes.

Fifteen healthy control subjects matched for age, gender, and ethnicity were recruited from the community by advertisement. None of the healthy control subjects had either a history of present or prior psychiatric disorder or had ever used any psychotropic medication as determined by SCAN interviews.

Four patients had prior ($n = 3$) or present ($n = 1$) use of antidepressant medication (in all cases selective serotonin reuptake inhibitors). Current use of benzodiazepines was allowed, albeit not on the day of the PET scans. Except for one, none of the patients used any drugs of abuse or fulfilled ICD-10 or DSM IV criteria for either drug abuse or drug dependence by the time of inclusion. None of the healthy controls or any of the patients had a history of significant head injury or non-psychiatric disorder. Both healthy controls and patients had a normal neurological interview and examination.

Magnetic Resonance Imaging

High-resolution 3D T1-weighted, sagittal, spoiled gradient echo scans (MPRAGE) of the head (TI/TE/TR = 800/3.93/1540 ms, flip angle 9°; matrix: 256 × 256; 192 slices) using an eight-channel head array coil were acquired in all subjects on a 3 T TRIO scanner (Siemens, Erlangen, Germany) at the MR department of the Copenhagen University Hospital, Hvidovre, Denmark.

[^{18}F]altanserin PET Studies

Radiosynthesis and administration. The radiosynthesis of [^{18}F]altanserin was according to the method described previously by Lemaire *et al* (1991). Quality control was performed using thin-layer chromatography and high-performance liquid chromatography (HPLC). The absence of residual solvents (methanol, THF, and DMSO) in the final formulation was confirmed by ^{1}H NMR. For each PET study, 0.3–3.5 GBq of [^{18}F]altanserin was produced with a radiochemical yield greater than 95% and a mean specific activity of 52.4 ± 34.0 GBq/μmol. Catheters were inserted in both cubital veins for tracer infusion and blood sampling, respectively. [^{18}F]altanserin was administrated as a combination of a bolus injection followed by continuous infusion to obtain steady state of the tracer in blood and tissue. The bolus-infusion ratio was 1.75 h, as previously described (Pinborg *et al*, 2003). Subjects received the maximum dose of 3.7 MBq/kg body weight [^{18}F]altanserin.

Imaging. PET scans were acquired in tracer steady-state conditions with an 18-ring GE-Advance scanner (GE, Milwaukee, WI, USA), operating in 3D-acquisition mode, producing 35 image slices with an interslice distance of 4.25 mm. The total axial field of view was 15.2 cm with an approximate in-plane resolution down to 5 mm. During steady state, the fraction of unmetabolized tracer in venous plasma was determined at five time points using HPLC analysis. Reconstruction, attenuation, and scatter correction procedures were conducted according to Pinborg *et al* (2003).

Ninety minutes after the bolus injection of [^{18}F]altanserin, the subjects were placed in the scanner. Subjects were aligned in the scanner using a laser system so that the detectors were parallel to the orbitomeatal line and positioned to include the cerebellum in the field of view using a short 2 min transmission scan. An individual head holder was made to ensure relative immobility. All subjects were scanned in a resting state. A 10-min transmission scan was obtained for correction of tissue attenuation using retractable ^{68}Ge/^{68}Ga pin sources. The transmission scans were corrected for tracer activity by a 5-min emission scan performed in 2D mode. Dynamic 3D emission scans (five frames of 8 min) were started 120 min after tracer administration.

Data were reconstructed into a sequence of $128 \times 128 \times 35$ voxel matrices, each voxel measuring $2.0 \times 2.0 \times 4.25$ mm, with software provided by the manufacturer. A 3D reprojection algorithm with a transaxial Hann filter (6 mm) and an axial ramp filter (8.5 mm) was applied. Corrections for dead-time, attenuation, and scatter were performed.

Blood samples. Five venous blood samples were drawn at mid-scan times 4, 12, 20, 28, and 36 min after starting the dynamic scanning sequence. The samples were immediately centrifuged, and 0.5 ml of plasma was counted in a well counter for determination of radioactivity. Three of the five blood samples drawn at 4, 20, and 36 min were also analyzed for percentage of parent compound ([^{18}F]altanserin) using reverse-phase HPLC following the procedure described by Pinborg *et al* (2003).

In addition, the free fraction of [^{18}F]altanserin in plasma, f_1, was estimated using equilibrium dialysis, following a modified procedure by Videbaek *et al* (1993). The dialysis was performed using Teflon-coated dialysis chambers (Harvard Bioscience, Amika, Holliston, MA, USA) with a cellulose membrane that retains proteins $>10\,000$ Da. A small amount of [^{18}F]altanserin (approximately 1 MBq) was added to 10 ml plasma samples drawn from the subjects. A 500 µl portion of plasma was then dialyzed at 37°C for 3 h against an equal volume of buffer, since pilot studies had shown that 3 h equilibration time yielded stable values. The buffer consisted of 135 mM NaCl, 3.0 mM KCl, 1.2 nM $CaCl_2$, 1.0 mM $MgCl_2$, and 2.0 mM phosphate (pH 7.4). After the dialysis, 400 µl of plasma and buffer were counted in a well counter, and f_1 of [^{18}F]altanserin was calculated as the ratio of $DPM_{buffer}/DPM_{plasma}$.

Data Analysis

MR/PET co-registration. PET images and magnetic resonance images (MRIs) were co-registered using a Matlab (Mathworks Inc., Natick, MA, USA)-based program (Willendrup *et al*, 2004), where PET images and MRIs are brought to fit through manual translation and rotation of the PET image with subsequent visual inspection in three planes (Adams *et al*, 2004).

Volumes of interest and partial volume correction. Volumes of interest (VOIs) were automatically delineated on each individual's transaxial MRI slices in a strictly user-independent manner (Svarer *et al*, 2005). With this approach, a template set of 10 MRIs is automatically co-registered to a new subject's MRI. The identified transformation parameters are used to define VOIs in the new subject MRI space, and through the co-registering these VOIs are transferred onto the PET images. The investigated regions included frontal cortex (consisting of orbitofrontal, medial inferior frontal, and superior frontal subregions), anterior cingulate, posterior cingulate, insula, superior temporal cortex, medial inferior temporal cortex, sensory motor cortex, parietal cortex, occipital cortex, putamen/pallidus, thalamus, caudate nucleus, and cerebellum. For normalization purposes (see below), a global neocortical region was created for each subject. It consisted of a volume-weighted average of the binding potentials from the cortical regions listed above.

To enable partial volume correction of the PET data, MRIs, corrected for RF inhomogeneities using the N3 software (Sled *et al*, 1998), were segmented into gray matter, white matter, and cerebrospinal fluid tissue classes using SPM2 (Wellcome Department of Cognitive Neurology, London, UK). Partial volume correction was performed according to Quarantelli *et al* (2004). The white matter value was extracted as the mean voxel value from a predominantly white matter VOI (mid-brain) in the uncorrected PET image. As the MRIs and PET images have been co-registered, it is possible to calculate the number of gray matter voxels in native subject space for each VOI, and this is reported as the gray matter volume for the VOI.

Quantification of the 5-HT$_{2A}$ receptor binding. The outcome parameter was the binding potential of specific tracer binding (BP_P). The cerebellum was used as a reference region, since it represents nonspecific binding only (Pinborg *et al*, 2003). In steady state, BP_P is defined as

$$BP_P = \frac{C_{ROI} - C_{Reference}}{C_{Plasma}} = f_1 \frac{B_{max}}{K_d}\ (\mathrm{ml/ml})$$

where C_{ROI} and $C_{Reference}$ are steady-state mean count density in the VOI and in the reference region, respectively, C_{Plasma} is the steady-state activity of non-metabolized tracer 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.

Statistics

Between-group (patients, controls) comparisons of all reported outcome measures were performed using parametric analysis after verifying that the data were normally distributed according to the Kolmogorov–Smirnov test. *P*-values from unpaired two-tailed *t*-test were reported; $p = 0.05$ was employed as the level of significance. *Post hoc* linear regression analysis was performed with caudate 5-HT$_{2A}$ receptor binding as the dependent variable and PANSS scores and age as independent variables. All analyses were performed using the statistical software SAS 9.1.

RESULTS

As shown in Table 1, no significant differences were observed in age, body mass index, injected dose, plasma free fraction, specific radioactivity of [^{18}F]altanserin, and nonspecific binding between the two groups.

As illustrated in Table 2, two-tailed unpaired *t*-test revealed no between-group differences in 5-HT$_{2A}$ BP$_P$ either in any of the cortical regions or in the thalamus or putamen. However, schizophrenic patients displayed a significantly higher 5-HT$_{2A}$ BP$_P$ in the caudate nucleus than controls (0.72 ± 0.15 *vs* 0.52 ± 0.27, $p = 0.02$, uncorrected). Exclusion of the subjects with prior antidepressant treatment (four patients) and illegal drug use (one patient) and their matched controls did not alter these results.

In the patient group, the positive, negative, and general symptom scores as assessed with PANSS were 18.6 ± 5.0, 20.6 ± 6.6, and 36.0 ± 7.1, respectively. *Post hoc* linear regression analysis with adjustment for age did not reveal any significant relationship between caudate 5-HT$_{2A}$ receptor binding and positive, negative, or general PANSS scores. Further, there was no statistically significant group difference in the ratio between uncorrected and partial volume-corrected BP$_p$ values in the caudate (0.4 ± 0.1 *vs* 0.5 ± 0.2 in controls, $p = 0.57$).

There were no between-group differences in total gray matter volume in the caudate (2.3 ± 0.3 *vs* 2.3 ± 0.2 ml in controls, $p = 0.49$) or in any of the other regions of interest (data not shown).

DISCUSSION

The number of antipsychotic-naive first-episode schizophrenic patients in our study is so far the largest sample examined. Earlier studies have included 6 (Ngan *et al*, 2000), 7 (Trichard *et al*, 1998), 10 (Okubo *et al*, 2000), and 10 (Lewis *et al*, 1999) patients. Our data are in agreement with most of the previously published PET studies (Lewis *et al*, 1999; Okubo *et al*, 2000; Trichard *et al*, 1998) where no significant difference in cortical 5-HT$_{2A}$ receptor binding was found in schizophrenic patients as compared to healthy controls. The data of Lewis *et al* (1999) were later reanalyzed with a voxel-based approach (Verhoeff *et al*, 2000) that confirmed the outcome of the region-based analysis. In contrast, Ngan *et al* (2000) reported a decreased 5-HT$_{2A}$ binding in the frontal cortex of six neuroleptic-naive schizophrenic subjects, and such a difference was also observed in a recent PET study of six subjects with elevated risk of developing schizophrenia (Hurlemann *et al*, 2005).

Despite some inconsistency in post-mortem data, the majority of post-mortem studies suggest a decreased cortical 5-HT$_{2A}$ receptor binding in patients with schizophrenia. This is in contrast to the outcome of most other *in vivo* studies, including our own. This discrepancy might be caused by the influence of antipsychotic drug treatment and cause of death in the studies of post-mortem brain tissues. Suicide as a cause of death has been associated with increased post-mortem 5-HT$_{2A}$ receptor density, especially in younger cohorts (Oquendo *et al*, 2006). At the same time, treatment with antipsychotic drugs that antagonize the 5-HT$_{2A}$ receptor decreases levels of expression of the receptor (for a review, see Dean, 2003). These factors are therefore important to take into account when interpreting reports of 5-HT$_{2A}$ receptor levels in post-mortem tissue from schizophrenic patients. *In vivo* PET data from antipsychotic-naive patients are spared from such confounders and might be more reliable in assessing receptor regulations in schizophrenia. On the other hand, autoradiographic post-mortem studies, in contrast to PET imaging, do allow for detection of differences in receptor binding within cortical cell layers. In conclusion, at present no firm conclusions can be made on cortical 5-HT$_{2A}$ receptor binding in patients with schizophrenia.

Four of the patients in the present study had prior ($n = 3$) or present ($n = 1$) use of antidepressant medication and one patient had illegal drug use by the time of inclusion. However, a *post hoc* analysis, performed after removing these patients and their controls from the analysis, did not change the results.

In patients with schizophrenia, we found an increased 5-HT$_{2A}$ receptor binding in the caudate nucleus, whereas no differences were seen in the thalamus or putamen. Owing to lack of selectivity of the radioligands employed in earlier studies, this is the first study to assess *in vivo* subcortical 5-HT$_{2A}$ receptor binding in schizophrenic patients. The 5-HT$_{2A}$ receptor density in subcortical brain regions is only modest, and accordingly for those brain regions a larger sample is required to exclude type II errors (Haugbøl *et al*, 2007). Furthermore, no relationship between severity of psychotic symptoms assessed with PANSS and caudate 5-HT$_{2A}$ receptor binding could be established. For the above reasons and since no corrections for multiple comparisons were made, we consider our finding of an increased 5-HT$_{2A}$ receptor binding in the caudate nucleus in schizophrenic patients as preliminary.

To assess any eventual regional pattern differences in further detail, we also took an additional approach. We and others have observed that cerebral 5-HT$_{2A}$ receptor binding displays a high degree of autocorrelation, so that a large fraction of the interindividual variability can be explained by a factor difference. To assess the subcortical binding

Table 1 Demographic and Scan Data

Parameter	Schizophrenic patients ($n = 15$)	Control subjects ($n = 15$)	*P*-value
Age (years)	27.5 ± 4.5	28.5 ± 5.7	NS (0.60)
BMI	23.7 ± 2.5	23.4 ± 2.2	NS (0.77)
Injected dose (MBq)	271 ± 45	278 ± 57	NS (0.70)
Specific activity	52.0 ± 36.9	52.8 ± 32.1	NS (0.95)
Free fraction (%)	0.36 ± 0.19	0.37 ± 0.16	NS (0.94)
Nonspecific binding	1.6 ± 0.3	1.6 ± 0.5	NS (0.83)

BMI = body mass index (body weight (kg)/height^2 (m^2)).

Table 2 Partial Volume-Corrected Binding Potentials of the Specific [^{18}F]altanserin Binding (BP_P) in Regions of Interest of Patients and Controls

Region	Schizophrenic patients	Control subjects	*P*-value
Neocortex	2.8 ± 0.6	2.7 ± 0.9	NS (0.60)
Frontal cortex (total)	2.9 ± 0.6	2.7 ± 0.9	NS (0.51)
Orbitofrontal cortex	2.7 ± 0.6	2.5 ± 0.8	NS (0.44)
Medial inf. frontal cortex	3.0 ± 0.6	2.8 ± 0.9	NS (0.52)
Superior frontal cortex	3.0 ± 0.6	2.8 ± 0.9	NS (0.54)
Anterior cingulate	2.4 ± 0.5	2.2 ± 0.8	NS (0.48)
Posterior cingulate	2.6 ± 0.6	2.3 ± 0.7	NS (0.36)
Insula	2.0 ± 0.4	1.9 ± 0.6	NS (0.47)
Medial inf. temp. cortex	2.6 ± 0.6	2.5 ± 0.8	NS (0.55)
Sup. temp. cortex	2.7 ± 0.5	2.5 ± 0.8	NS (0.45)
Parietal cortex	3.3 ± 0.7	3.1 ± 1.0	NS (0.63)
Sens. mot. cortex	2.8 ± 0.6	2.6 ± 0.8	NS (0.43)
Occipital cortex	2.8 ± 0.6	2.9 ± 0.9	NS (0.75)
Thalamus	0.6 ± 0.1	0.5 ± 0.2	NS (0.15)
Putamen	0.7 ± 0.2	0.6 ± 0.3	NS (0.37)
Caudate	0.7 ± 0.1	0.5 ± 0.3	0.02

relative to the cortical binding, we normalized the subcortical regions with a volume-weighted average of cortical BP_P and evaluated the within-group difference. Using these normalized values, the difference in caudate values turned out to be even more significant ($p = 0.001$).

If confirmed, an increase in 5-HT$_{2A}$ receptor levels in schizophrenic patients could support a direct role of blockade of striatal 5-HT$_{2A}$ receptors in the mechanisms of action of a number of second-generation antipsychotics—in addition to the assumed indirect effects via modulation of cortical as well as striatal dopamine activity by 5-HT$_{2A}$ receptor blockade (Glenthoj *et al*, 1999; Meltzer *et al*, 2003; Svensson *et al*, 1995). An independent role of 5-HT$_{2A}$ receptor blockade in the mechanisms of action of second-generation antipsychotics is also supported by data demonstrating an association between polymorphisms in the promoter and coding regions of the 5-HT$_{2A}$ receptor gene and schizophrenia and/or the response to treatment with clozapine (Abdolmaleky *et al*, 2004; Arranz *et al*, 1998a, b; Masellis *et al*, 1998). Furthermore, second-generation antipsychotics are also effective as an add-on to treatment with selective serotonin reuptake inhibitors in patients with obsessive–compulsive disorder (Denys *et al*, 2007; Skapinakis *et al*, 2007), and we have previously found increased 5-HT$_{2A}$ receptor binding in the caudate nuclei of patients with this disease (Adams *et al*, 2005). The finding of increased 5-HT$_{2A}$ receptor binding in the caudate nuclei in patients with obsessive–compulsive disorder as well as in patients with schizophrenia may suggest a common pathophysiological mechanism in agreement with the frequent occurrence of obsessive–compulsive symptoms in schizophrenic patients (Kayahan *et al*, 2005a, b). If an increase in striatal 5-HT$_{2A}$ receptor binding is confirmed, the effects could still, however, be mediated through interactions with the dopaminergic system.

An increased 5-HT$_{2A}$ receptor binding in the caudate nucleus, as suggested by the preliminary data in the present study, might alternatively result from a compensatory upregulation of 5-HT$_{2A}$ receptors in response to altered serotonin levels. In addition, a defect in the medial raphe-cortico-striatal serotonergic circuit has been suggested to result in disinhibition of the mesolimbic DA system, a mechanism likewise suspected to play an important role in the pathophysiology of schizophrenia (Abi-Dargham *et al*, 1997). Because of a paradoxical regulation of the serotonin 5-HT$_{2A}$ receptor (Gray and Roth, 2001), antagonism would lead to downregulation of the receptor, thereby normalizing its levels. The changes in striatal 5-HT$_{2A}$ receptor density in schizophrenia are in accordance with the post-mortem findings by Joyce *et al* (1993). By contrast, Matsumoto *et al* (2005) did not find differences in striatal 5-HT$_{2A}$ receptor density between schizophrenic subjects and controls; likewise, no change in striatal 5-HT$_{2A}$ receptor binding was seen in a smaller study of six subjects at risk of schizophrenia (Hurlemann *et al*, 2005).

CONCLUSION

The present study is the first PET study exploring striatal as well as cortical 5-HT$_{2A}$ receptor binding in first-episode antipsychotic-naive schizophrenic patients. It is also the largest *in vivo* study on 5-HT$_{2A}$ receptors in those patients until now. We find no difference in the cortical regions or in the thalamus or putamen between the two groups, whereas an increased 5-HT$_{2A}$ receptor binding is detected in the caudate nucleus in 15 first-episode antipsychotic-naive schizophrenic patients when compared to age- and gender-matched healthy control subjects. This supports a direct or an indirect role of striatal 5-HT$_{2A}$ receptors in the pathophysiology of schizophrenia and in the mechanisms of action of many atypical antipsychotics. Further studies are, however, needed to explore the relation of subcortical and cortical 5-HT$_{2A}$ receptor activity to psychopathology, information processing, and other neurobiological measures.

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The authors have nothing further to disclose.

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