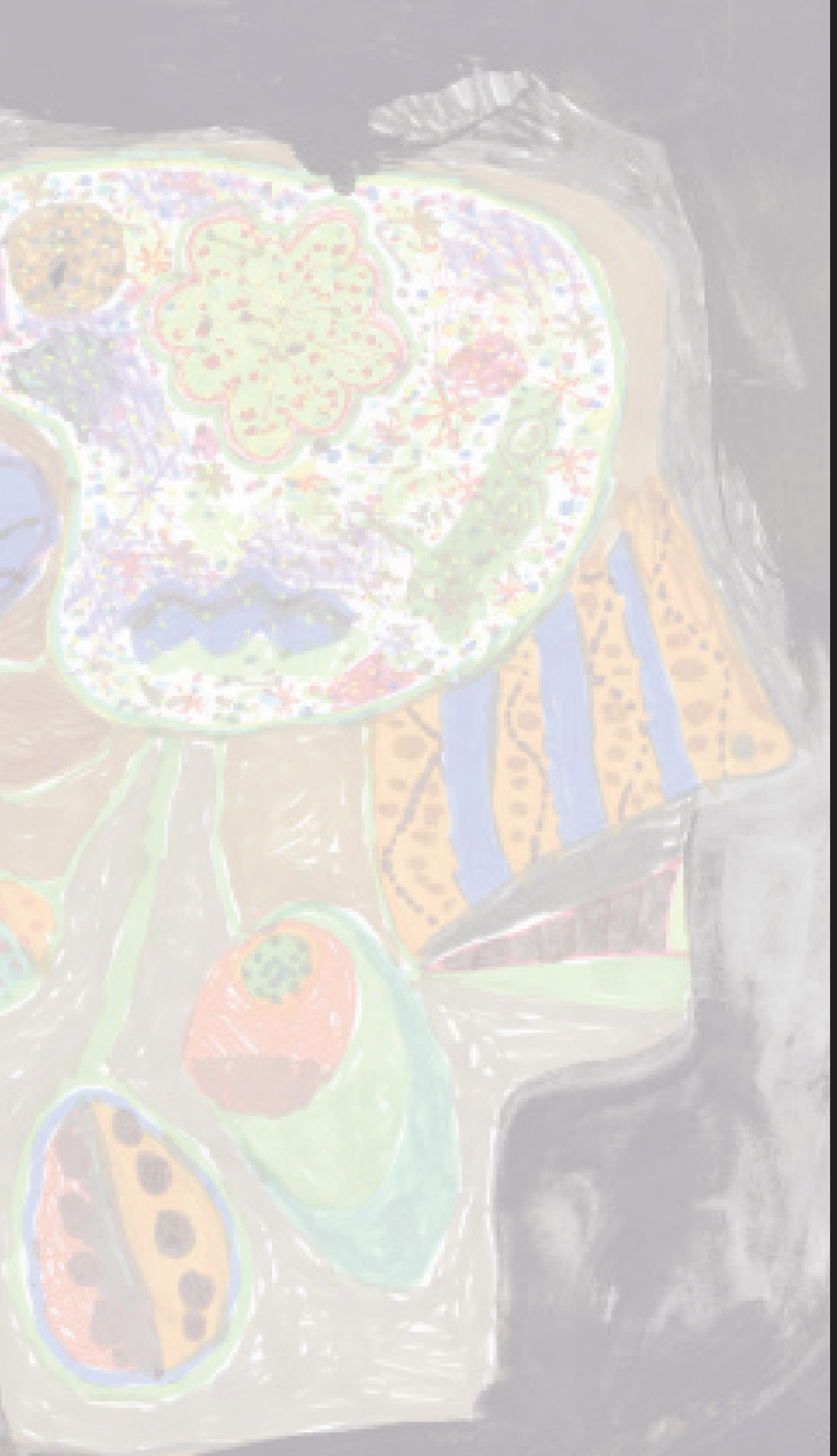


Printed in Denmark · www.lemann.dk

S. Haugbøl

Imaging brain serotonin 2A receptors: Methodological and genetic aspects and involvement in Tourette's syndrome

Imaging brain serotonin 2A receptors: Methodological and genetic aspects and involvement in Tourette's syndrome

Steven Haugbøl

FACULTY OF HEALTH SCIENCES
UNIVERSITY OF COPENHAGEN

PhD thesis by

Steven Haugbøl, MD

From the
Neurobiology Research Unit
The Neuroscience Center
Department of Neurology
Rigshospitalet, Copenhagen University Hospital
Denmark
Steven.haugbol@nru.dk

Imaging brain serotonin 2A receptors: Methodological and genetic aspects and involvement in Tourette's syndrome

Supervisors

- Professor *Gitte M. Knudsen*, MD, Dr. Med., Department of Neurology, Neurobiology Research Unit, Rigshospitalet, Copenhagen University Hospital, Denmark
- Associate professor *Lis Hasholt*, Dr. Med., Department of Medical Biochemistry and Genetic, Section of Neurogenetics, University of Copenhagen, Denmark

Review committee

- Associate professor *Erling T. Mellerup*, Dr. Med. Sci. (Chairman), Department of Pharmacology, Laboratory of Neuropsychiatry, University of Copenhagen, Denmark
- Professor *Juha O. Rinne*, MD, Dr. Med., Turku PET Centre, Turku University Hospital, Finland
- Consultant *Kirsten R. Müller-Vahl*, MD, Dr. Med., Department of Clinical Psychiatry, Medical School Hannover, Germany

Cover page

"Andres Hjerner" by Tal R and Yunus

The *Viva voce* examination takes place Friday, February 2, 2007, 2 pm at Rigshospitalet, Copenhagen, in Auditorium 1, Building 44.

"Doctors are men who prescribe medicines of which they know little, to cure diseases of which they know less, in human beings of whom they know nothing"

Voltaire, 1694 - 1778

Acknowledgements

The present work was carried out during my appointment at the Neurobiology Research Unit at Department of Neurology in the Neuroscience Centre of Rigshospitalet, Copenhagen University Hospital.

The study would not have been possible without support from many worthy colleagues, coauthors and permanent staff members. A special thank you to *Inge Møller, Karin Stahr, Dorthe Givard and Pia Farup* for technical and administrative assistance. Furthermore, I am deeply grateful to my principal supervisor *Gitte Moos Knudsen* who has shown me the value of scientific enthusiasm and professional presentation skills. Her kind guidance has always been of high intellectual standard and her philosophy always untiring and positive. Likewise, I would like to express thanks to co-supervisor *Lis Hasholt* and technician *Lisbeth Olsen* and their colleagues for their valuable advice and introduction to genetic laboratory work. To all I would like to express my gratitude for the cooperation, many fruitful discussions and pleasant fellowship with the hope of continuation of the established cooperation in the future. Also, I would like to express my deepest gratitude to the people with Tourette's syndrome and healthy volunteers who altruistically participated in the studies. In addition, a particular thank you to *Mary M. Robertson* for her kind hospitality and clinical training at the Institute of Neurology, Queen Square, London. Moreover, I would like to express my gratitude to the foundations from which I received financial support:

Rigshospitalet Research Council, A.P. Møller and wife Chastine Mc-Kinney Møller Foundation, The John and Birthe Meyer Foundation, The Medical Research Fund of Copenhagen, Ludvig and Sara Elsass Foundation, Hørslev Foundation, Director Jacob Madsen & Wife Olga Madsen's Foundation, The Augustinus Foundation, The Beckett Foundation, Foundation for Neurological Research, H:S Research Council.

And finally with love to my wife and family.

Gentofte, October 2006
Steven Haugbøl

English summary

Extensive research of the serotonergic system shows that serotonin mediates a number of different physiological functions in the brain, e.g. sleep, feeding, pain control, thermoregulation, cognition, emesis and sexuality. Moreover, alterations of the serotonergic neurotransmission are implicated in the pathophysiology of several neurological and psychiatric disorders including depression, anxiety disorder including obsessive-compulsive disorder, schizophrenia, migraine and Tourette's syndrome. As our knowledge about the significance of the serotonin system grows, new questions are asked about potentially important features in serotonin signaling such as function, regulation of transmission and interaction with other transmitter systems. Finding answers to these inevitable questions to continue progress in serotonergic research necessitates development of new approaches and careful validation of existing ones. Several milestones in neurobiological research attribute to the principal methods of neurotransmitter and receptor binding analysis. The nuclear medicine imaging technique positron emission tomography (PET) use radiolabeled drugs with specific properties to enable *in vivo* imaging of brain receptors and transporters in the living brain. This method is a powerful tool to measure biological processes in different brain regions and to some extent, it overcomes limitations of postmortem, platelet and genetic studies in analyzing brain receptor functions.

The first part of this thesis investigates the reliability and power of using the positron emission tomography method to study the post-synaptic serotonin 2A receptor with the radiotracer [^{18}F]altanserin. The investigations establish reliable measurements of [^{18}F]altanserin for imaging and quantification since repeated positron emission tomography scans show high intraclass correlation, at least between brain regions with high tracer binding. In addition, sample size calculations identify the required number of people in studies between independent groups and in paired design. Likewise, the results shows that corrections for spill–over effect of white matter and cerebral spinal fluid in brain regions and adjustments for explaining variables to the binding potential decrease the sample sizes to generate trustworthy conclusions.

Part two of the thesis investigates the possible genetic influence on the [^{18}F]altanserin binding to the serotonin 2A receptor in the living brain. A single nucleotide substitution polymorphism and a amino acid transition polymorphism of the serotonin 2A receptor gene and two variable number of tandem repeats in the serotonin transporter gene were associated with radiotracer binding to the serotonin 2A receptor binding ([^{18}F]altanserin). This investigation shows possible influence of the two tandem repeats in the serotonin transporter gene, thus confirming previous studies showing that differential expression of the serotonin transporter can consequently underlie regulation of the serotonergic neurotransmission.

Part three reviews the possible implication of the serotonin system in Tourette's syndrome and describes a PET study of Tourette's syndrome patients with the radiotracer. This approach revealed an overall increased tracer binding in the brain compared to healthy controls. Several lines of hypotheses link the serotonin system to be involved in Tourette's syndrome and this result adds further to the discussion of the pathophysiology of the disorder.

In summation, knowledge of reproducibility and sensitivity of the [^{18}F]altanserin PET method when including partial volume correction and adjustment for covariates like age, neuroticism and polymorphisms in the serotonin transporter gene is expected to improve trial design and provide more consistent PET results. This will improve our knowledge of the etiology of neurological and neuropsychiatric disorders and progress the development of effective pharmacotherapy. Furthermore, with reference to thirty years of serotonin 2A receptor research where detailed information of regional and cellular localization and potential roles of the physiology has been achieved, we now add further to the knowledge of the complex roles of regulation of this receptor by demonstrating associations between polymorphisms in the serotonin transporter gene and 5-HT_{2A} receptor binding in the living brain of healthy volunteers. Our findings of an increased density of serotonin 2A receptors in Tourette's syndrome patients support previous studies showing involvement of the serotonin system in this disorder, thus demonstrating a probable connection to obsessive-compulsive behavior and interplay with the dopaminergic system which possibly dominates the pathophysiology of Tourette's syndrome.

Dansk resumé (Danish summary)

Afhandlingen omhandler undersøgelser af serotonin 2A receptoren i hjernen hos det levende menneske. Det serotonerge neurotransmittersystem er involveret ved en række neurologiske og psykiatriske sygdomme, herunder depression, skizofreni, angst, obsessiv-kompulsiv sygdom og Tourette's syndrom. Serotoninsystemet er også involveret ved træk i den menneskelige adfærd, som impulsivitet, aggressivitet, søvn og appetit.

Den første del af afhandlingen omfatter en metodemæssig evaluering af reproducerbarheden af bolus-infusionsmetoden med positron emissionstomografi (PET)-liganden [^{18}F]altanserin. Derudover inddrages PET-data fra 84 raske personer til at estimere den nødvendige stikprøvestørrelse i lignende PET-studier. I anden del af afhandlingen undersøges eventuelle associationer mellem hjernens serotonin 2A receptorbinding og tre hyppigt forekommende polymorfier i serotonin 2A receptoren, og to ligeledes hyppigt forekommende polymorfier i serotonin transporteren med henblik på eventuelt at forklare den observerede variabilitet mellem personer. I afhandlingens sidste del beskrives en undersøgelse af hjernens serotonin 2A receptorbinding hos patienter med Tourette's syndrom og hos raske forsøgspersoner.

Sammenfattende vises det, at bolus-infusionsmetoden med [^{18}F]altanserin-PET er meget reproducerbar, særlig i større regioner med høj koncentration af serotonin 2A receptorer. Det påvises endvidere, at metoden er tilstrækkelig sensitiv til at påvise selv mindre forskelle mellem to grupper, særlig i hjerneregioner med høj receptorkoncentration og når der korrigeres for eventuelle forskelle i hjerneatrofi og variable som alder, neuroticisme og genetiske polymorfier. Endvidere viser undersøgelserne, at to funktionelle variable tandem repeats i serotonin transporteren er forbundet med forskelle i serotonin 2A receptorbindingen, hvorimod polymorfier i serotonin 2A receptorgenet overraskende ikke har nogen effekt. Endelig viser afhandlingen, at serotonin 2A binding er øget hos patienter med Tourette's syndrom i forhold til raske kontrolpersoner. Sidstnævnte fund giver anledning til overvejelser om receptorens funktionelle betydning i relation til symptomerne, om end der ikke kan påvises en association mellem sværheden af tics og serotonin 2A bindingen.

LIST OF CONTENTS

List of abbreviations

[11C]DASB	[11C]-3-amino-4-(dimethylaminomethyl-phenylsulfanyl)-benzonitrile
[^{11}C]WAY	[11C]WAY 100635
[^{123}I]β-cit	[123I](2b-carboxymethoxy-3b-[4-odophenyl]tropane)
102 *t/c*	Polymorphism in 5-HT_{2A} receptor gene
-1438 *g/a*	Polymorphism in the 5-HT_{2A} receptor gene
452 *His/Tyr*	polymorphism in the 5-HT_{2A} receptor gene
5-HIAA	5-hydroxy-indoleacetic acid
5-HT	5-hydroxytryptamine, serotonin.
5-HT_{2A}	Serotonin 2A receptor
5-HTT	serotonin transporter (~ serotonin reuptake site)
5-HTTLPR	Polymorphism in the promoter region of the serotonin transporter
ADHD	Attention deficit hyperactivity disorder
ANOVA	Analyses of variance
BBB	Blood brain barrier
B_{max}	Available receptor site concentration (nM)
BMI	Body mass index
BP_1	Binding potential ($B_{MAX}/K_{d;}$ unit less)
$c_{cerebellum}$	Bound radioactivity concentration in cerebellum, reference tissue (MBq/ml)
c_{plasma}	Concentration of tracer plasma radioactivity (MBq/ml)
c_{ROI}	Bound radioactivity concentration in a region of interest (MBq/ml)
CSF	Cerebral spinal fluid
CSTC	Cortico-striatal-thalamo-cortical
DOI	(±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride
f_1	The free fraction of tracer in plasma (unit less or %)
FDG	Fluorodeoxyglucose
fMRI	Functional magnetic resonance imaging
GPCR	G-protein coupled receptor
HAMD	Hamilton depression scale
HPLC	High performance liquid chromatography
HTR2A	serotonin 2A receptor gene
K_d	Equilibrium dissociation constant
LSD	Lysergic acid diethylamide
MDMA	3,4-methylenedioxymethamphetamine
Miredif	Minimum expected difference
MRI	magnetic resonance image
OCD	Obsessive-compulsive disorder
PCR	Polymerase chain reaction
PET	Positron emission tomography
PVE	Partial volume estimation
ROI	Region of interest
SLC6A4	serotonin transporter gene
SPECT	Single photon emission computed tomography
SPM	Statistical parametric mapping
SSRI	Selective serotonin reuptake inhibitor
TRP	Tryptophan
TS	Tourette's syndrome
V_d	General volume of distribution
$V_{d(ROI)}$	Volume of distribution in a specific region of interest
$V_{d(NS)}$	Volume of distribution in cerebellum, reference tissue
VNTR-2	Polymorphism in the second intron of the serotonin transporter
Y-BOCS	Yale-Brown obsessive-compulsive scale
YGTSS	Yale global tic severity score

List of publications

This thesis is based on the following papers.

I. Haugbol S, Pinborg LH, Arfan HM, Frokjaer VM, Madsen J, Dyrby TB, Svarer C, Knudsen GM. Reproducibility of 5-HT(2A) receptor measurements and sample size estimations with [(18)F]altanserin PET using a bolus/infusion approach. *European Journal of Nuclear Medicine and Molecular Imaging. 2006 Dec 29.*

II. Haugbøl S, Pinborg LH, Frøkjær V, Erritzøe D, Marner L, Hasselbalch SG, Hasholt L, Svarer C, Paulson O, Holm S, Knudsen GM. A serotonin transporter polymorphism influence *in vivo* 5-HT_{2A} receptor binding in the brain. *Submitted.*

III. Haugbol S, Pinborg LH, Regeur L, Hansen ES, Bolwig TG, Nielsen FA, Svarer C, Skovgaard LT, Knudsen GM. Cerebral 5-HT2A receptor binding is increased in patients with Tourette's syndrome. *The International Journal of Neuropsychopharmacology. 2006 Feb 28.*

Introduction

General introduction

The main focus of this thesis is the visualization of cerebral serotonin 2A (5-HT_{2A}) receptors in the living human brain with the radiotracer [^{18}F]altanserin and positron emission tomography (PET).

The themes of the thesis are:

i. Reproducibility of bolus plus infusion [^{18}F]altanserin-PET measurements.

ii. Required sample sizes in [^{18}F]altanserin-PET studies with relation to explaining covariates of the binding potential and partial volume estimation.

iii. The relation between in vivo 5-HT_{2A} receptor binding and polymorphisms in the 5-HT_{2A} receptor and serotonin transporter genes.

iv. 5-HT_{2A} receptor binding in patients with Tourette's syndrome patients as compared to healthy volunteers.

The mammalian brain is among the most complex organs in terms of biochemistry and anatomy. It is recognized that the brain is responsible for a majority of complex neuropsychiatric diseases, but the relationship between function and behavior remains for the most part elusive. Geneticists suggest that at least half of the human genes code for products that are expressed in the brain [1]. These genes may influence development, daily skills and aspects of the personality. Of the approximately 4000 genetic diseases [2], around one in four involves the brain [3]. In addition to incalculable personal toll, annual economic costs of billions of euro, calculations from year 2003 show that all brain diseases together constitute around 35 percent of the European health burden assessed through disability-adjusted life years [4]. Our overall strategy is, by means of this research, to understand the normal and abnormal structure and activity of parts of the nervous system to accomplish reduction of the burden of neuropsychiatry diseases in the longer term.

Neurotransmitters which mediate the signal between nerve cells and receptors which conduct signal from neurotransmitters are altered in many neurological and psychiatric disorders and serve as targets for pharmacological intervention. Substances that act as neurotransmitters can roughly be categorized into three groups; amino acids (e.g.

glutamate and gamma-aminobutyric acid), petides (e.g. vasopressin and somatostatin) and monoamines (e.g. serotonin, dopamine and norepinephrine). In the serotonin system, the 5-HT_{2A} receptor along with the serotonin 1A (5-HT_{1A}) receptor and also the serotonin re-uptake (serotonin transporter; 5-HTT) site are the most frequently targeted sites of action for pharmacologic treatment [5,6] and thus also for radiopharmaceuticals [7].

Since the brain imaging technique PET was introduced three decades ago, it has flourished as a versatile tool in scientific studies of the living brain. In addition to *in vivo* imaging of cerebral blood flow and glucose metabolism, it allows measurements of specific receptors and neurotransmitter release which constitute a major part of the understanding of many neuropsychiatric diseases.

The introduction of this thesis addresses general aspects of the serotonin system, the 5-HT_{2A} receptor, Tourette's syndrome and PET.

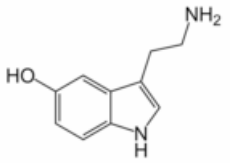
The serotonin system

Serotonin (5-hydroxytryptamine; 5-HT) was identified and isolated over 50 years ago. It was characterized as a vasoconstrictive substance in serum and subsequent studies demonstrated its presence in brain tissue. Today we know that 5-HT plays a major role as a neurotransmitter. It is implicated in various physiological functions, including sleep [8], appetite [9], pain control [10], thermoregulation [11], cognition [12], emesis [13] and sexuality [14].

Disturbed 5-HT function has been suggested for several neurological and psychiatric disorders, such as depression [15], anxiety disorders including obsessive-compulsive disorder [16,17], Tourette's syndrome [18], schizophrenia [19], addiction [20], migraine [21] and dementia [22]. In addition, previous studies has identified its implication in personality traits such as aggression dyscontrol [23], danger avoidance [24] and sensibility to stressful life events [25].

The serotonergic system is also a main target for pharmacological treatment of several neurological and psychiatric disorders. In particular, selective serotonin reuptake inhibitors (SSRI's) are used for treatment of depression and obsessive-compulsive disorder (OCD) [26,27]. Atypical antipsychotic such as risperidone and olanzepine which have high affinity for 5-HT_{2A} receptors are used in schizophrenia [28] and Tourette's syndrome [29], and triptans [30] which act as vasoconstrictors via the 5-$HT_{1B/1D}$ receptors are used in migraine.

Figure 1

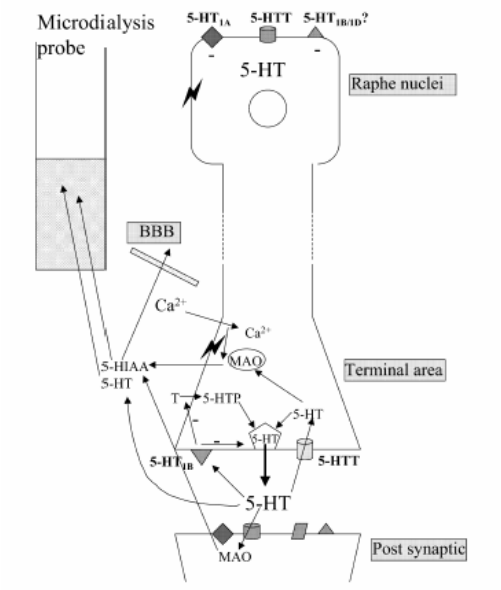


Molecule of 5-hydroxytryptamin (5-HT, or serotonin). 5-HT is a monoamine neurotransmitter that contains one amino group which connects to an aromatic ring by a two-carbon chain. It is synthesized from the essential amino acid tryptophan in nerve cells of the raphe nuclei in midbrain which distributes serotonin widely in the brain. It influences several fundamental physiological functions including sleep, feeding, pain control, thermoregulation, cognition, emesis and sexuality.

5-HT is synthesized from the essential amino acid tryptophan through a short metabolic pathway. Tryptophan crosses the blood brain barrier and membranes of nerve cell to hydroxylate into 5-hydroxytrytophan which again decarboxylate to 5-HT.

.

Figure 2



A simple illustration of the 5-HT pathway and factors that determine its function. Tryptophan distributes from the blood stream, is metabolized to 5-HT in nerve cells, and upon changes of membrane potential 5-HT releases into extra-neuronal space to mediate its actions on receptors. The action is primarily terminated by reuptake from the synapse by the 5-HTT. Degrading happens by the monoamine oxidase in

the presynaptic terminal whereas a product is 5-hydroxyindoleaceticacid. The 5-HT_{1A} and 5-HT_{1B} receptor inhibits processes in the transmission by negative feedback mechanisms. BBB: blood brain barrier, 5-HIAA: 5-hydroxyindoleaceticacid, T: Tryptophan, 5-HTP: 5-hydroxytryptophan, 5-HTT: serotonin transporter (5-HT reuptake mechanism). From Stenfors and Ross [31].

The neurons of the raphe nuclei in the dorsal part of midbrain and brainstem are the principal sources of 5-HT release. These neurons are grouped along the entire length of the brainstem. The endogenous 5-HT is being mediated through seven classes of 5-HT receptors classified on the basis of structure and functional properties where six of these are G-protein coupled. These classes are further subdivided, although 5-HT_4, 5-HT_6 and 5-HT_7 classes do not contain subclasses of receptors. The 5-HT_3 receptor is a ligand-gated ion channel resulting in a direct plasma membrane depolarization [32] (Table 1). In addition, sequence diversity has been provided by RNA editing and, further, numerous single-nucleotide polymorphisms (SNP's) and variable number of tandem repeats (VNTR's) exist in the serotonin receptor genes and the reuptake site for 5-HT.

Table 1

Receptor class	Subtypes
5-HT_1 (G-protein coupled)	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{1P}, 5-HT_{1S}
5-HT_2 (G-protein coupled)	5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}
5-HT_3 (ligand gated)	
5-HT_4 (G-protein coupled)	
5-HT_5 (G-protein coupled)	5-HT_{5A} and 5-HT_{5B}
5-HT_6 (G-protein coupled)	
5-HT_7 (G-protein coupled)	

5-HT mediates a wide range of physiological and pathophysiological functions by interacting with multiple receptors. Seven distinct families of 5-HT receptors have been identified and subpopulations have been described for several of these. At least 15 subpopulations have now been cloned.

The 5-HT receptors are located on the dendrites, cell bodies and presynaptic terminals of adjacent neurons all over the brain [33]. Serotonergic action is terminated primarily via uptake of 5-HT from the synapse. This takes place through the specific monoamine 5-HT reuptake transporter (5-HTT) on the presynaptic neuron [34]. The 5-HTT function is determined by many different regulatory factors, such as the composition of the plasma membrane [19], genotype [35], growth factors [20], interleukine [21], stress [22] and glucocorticoids [23]. These features make the 5-HTT a suitable target for drugs as well [24].

Positron emission tomography

The main strength of PET is its ability to measure physiological processes and measurements of receptor and transmitter changes in living healthy and diseased humans. However, its use as a diagnostic tool in clinical neurology and psychiatry has been limited because of much cheaper and available standard diagnostic tools. On the other hand, the measurement of neurotransmitter and neuroreceptor changes with PET is increasingly contributing to pathophysiological understanding, drug development and drug therapy.

Imaging of the serotonergic transmitter system in the living human brain has become possible by the use of radiolabeled tracers and PET, thus enabling conduction of studies to elucidate the possible dysfunction of the serotonergic system in the living brain. The huge potential of this approach has to be carefully evaluated to optimize the gain from quantitative data.

Here, we give special emphasis towards the 5-HT_{2A} receptor. Early studies of the this receptor involved the use of less selective 5-HT_{2A} receptor tracers, such as [^{18}F]setoperone and [^{11}C]N-methyl-spiperone. The more recently developed 5-HT_{2A} receptor tracer [^{11}C]MDL100907 [36] and [^{18}F]altanserin [37] yield a specific signal with a high target-to-background ratio. These two tracers possess similar binding characteristics [38], but quantification of [^{18}F]altanserin binding is complicated by the systemic production of radiolabeled metabolites that cross the blood-brain barrier. Several studies have tried to overcome the metabolite issue by sophisticated kinetic modeling where the kinetics of the radiolabeled compounds are taken into account [39]. Use of a bolus-infusion [^{18}F]altanserin protocol does, however, allow for a reliable subtraction of the non-specific binding of radiotracer [40]. Reproducibility of altanserin PET measurements with bolus administration has previously been evaluated in a test-retest study [41], but no thorough analysis of the individual sources of variation (plasma free fraction, non-specific binding, partial volume estimation, explaining variables, etc).

5-HT_{2A} receptors

The 5-HT_{2A} receptor is present in all cortical regions whereas the subcortical regions and hippocampus have lower density [42]. In striatum, the 5-HT_{2A} receptor is particularly found in striosomes [43] which control input from the cortical regions [44,45]. The majority of receptors are expressed by pyramidal neurons [46] and some are present in GABA'ergic interneurons [47]. These interactions regulate glutamate release in others pyramidal layers and the 5-HT release in different synapses and raphe nuclei [47]. Activation of 5-HT_{2A} receptors in frontal regions by agonists increases glutamate release [48]. Moreover, 5-HT_{2A} receptors antagonists induce changes in prefrontal dopamine release [49]. The dopaminergic system also shows 5-HT_{2A} receptors on ventral tegmental area and substantia nigra [50] implicating a role for the receptors regulation on dopamine pathways.

Given the potential relevance of the receptor function there is numerous clues about a role for 5-HT_{2A} in the pathophysiological function, such as some hallucinogens (e.g. LSD, DOI) are 5-HT_{2A} agonists and atypical antipsychotics are 5-HT_{2A} antagonists [51,52]. In recent years, a number of open-label and placebo controlled studies have

suggested that atypical antipsychotic drugs and some antidepressants (e.g., mirtazapine and mianserin) augment the clinical response to SSRI's in treatment-resistant patients [53,54]. One common feature of these agents is their ability to occupy 5-HT_2 receptors in the brain and to block 5-HT_{2A}-mediated responses [55]. Many antidepressants down-regulate 5-HT_{2A} receptors after repeated treatment [56], which, together, support a role for 5-HT_{2A} receptors in antidepressant drug action. These observations agrees with that the majority of post mortem studies in suicide victims suffering from major depressions have increased 5-HT_{2A} binding in the frontal cortex [57]. However, PET and SPECT studies in depressive patients have failed to confirm this finding [58-60].

To sum up, especially the prefrontal cortex is densely innervated by 5-HT axons and is highly enriched in various receptors, notable serotonin receptors e.g. 5-HT_{2A} receptors. Numerous studies indicates 5-HT_{2A} receptors are implicated in disorders like psychosis [61], anxiety and depression [62], addiction [20], obsessive-compulsive disorder and Tourette's syndrome [18,63] and personality traits of extreme ranges, and finally, memory [12,24].

5-HT_{2A} receptor signaling

The 5-HT_{2A} is a G protein-coupled receptor (GPCR), also known as a seven transmembrane receptor. GPCR family consists of a single polypeptide chain having an extracellular, transmembrane and intracellular domain. While some other types of receptors bind neurotransmitters to the membrane externally, the transmitters of GPCRs typically bind within the transmembrane domain. Upon specific transmitter binding, the conformation of the receptor can change and transmit the extracellular signal to the inside of the cell and trigger the activation of the G-protein [64]. The active G protein has the potential to induce various cascades of intracellular signaling and can cause a broad spectrum of cellular responses.

Even though the transduction of the signal through the membrane by the receptor is not completely understood, it is known that the inactive G-protein is bound to the receptor in its inactive state. Once the transmitter is recognized, the receptor shifts conformation and thus mechanically activates the G protein, which detaches from the receptor. The receptor can now either activate another G protein, or switch back to its inactive state [65,66].

The interaction of the agonists with the receptor has been described by the ternary complex model. This model involves the interaction between a tracer and its receptor: receptor + tracer ↔ receptor-tracer complex. Agonists have higher affinity to GPCR compared to uncoupled receptors and preferentially bind to the active conformation, thereby stabilizing the receptor complex and leading to increased cellular activity. Usually, distinction is made between high and low affinity conformational state, and it is assumed that the high affinity state is the active receptor that can either be coupled to or decoupled from the G-protein and the low affinity state is the inactive receptor. Antagonists will bind with equal affinity either state whereas agonists will preferentially bind to the high affinity state [67].

If a GPCR is exposed to their transmitter for a prolonged period of time signaling is then attenuated by GPCR internalization (desensitized). The key reaction of this

downregulation is two processes depending on the time scale of which it occurs. Short term regulations within seconds to minutes involve phosphorylation by cyclic adenosine monophosphate (cAMP) dependent proteins kinases (protein kinase A). The phosphorylation of the receptor is supposed to, consequently, bring it inside the cell were it can be dephosphorylated and then brought back. Long term regulation occurs within hours and days and involves degradation and probably decreased expression of the receptor [68,69]. Another action is that the phosphorylated receptor can be linked to arrestin molecules that prevent it from binding (and activating) G proteins, effectively switching it off for a short period of time [70]. Paradoxically, the 5-HT_{2A} receptor binding appears to downregulate not only upon chronic agonist stimulation but also after chronic treatment with antagonists [56].

5-HT system in neurodevelopment

The profound involvement of 5-HT in neurodevelopment supports a role for 5-HT in the etiology and/or pathophysiology of persistent developmental disorders and other brain disorders. 5-HT and its associated transporter and receptors are found in rats as early as day 12 and in humans by week 5 [71,72]. Much of the early expression of 5-HT appears to be related to its role as a growth factor and regulator of neuronal development [73]. Thus, in addition to functioning as a modulator of neural transmission, 5-HT has critical effects on neurogenesis, morphogenesis and synaptogenesis in the developing brain [74]. There is also evidence that SSRI's could involve developmental mechanisms, thus in adults, antidepressants promote neurogenesis in hippocampus [75,76]. Together with the influence of 5-HT in neurodevelopment, the observation of an effect of 5-HTT gene variants (5-HTTLPR and VNTR-2) on patterns of 5-HTT gene expression in the developing brain suggests influences on the risk of developing affective diseases [25,74]. Furthermore, progress in 5-HTT gene research supports that the variability of functional 5-HTT expression has a relevant function in neurodevelopment. Though, Lesch et al. (1996) [35] found that individuals with the short allele genotype in the 5-HTT had higher neuroticism score than individuals homozygous for the long variant. Likewise, a recent study on mice over expressing 5-HTT exhibits a low-anxiety phenotype probably secondary to low extracellular 5-HT [77].

5-HT_{2A} receptor and 5-HTT genes

The 5-HT_{2A} gene (HTR2A) spans over 20 kb and contains 3 exons separated by 2 introns. Genes encoding a number of the components involved in 5-HT neural transmission have been examined as possible contributors to the potentially relevant behaviors and disorders mentioned. For example, post mortem tissue from depressed [78] and schizophrenic [79] patients demonstrate altered mRNA expression of this receptor and meta-analysis associates the *c* allele of the 102 *c/t* single nucleotide polymorphism (SNP) in the HTR2A to schizophrenia [80]. The amino acid changing SNP 452 *His/Tyr* is localized in the coupling zone of the c-terminal region in the 5-HT_{2A} receptor. A meta-analysis found significant association between the allele 452 *Tyr* and poor response to clozapine [81]. This suggests that a possible conformational change can influence the drug target in 5-HT_{2A} receptor.

The 5-HTT gene (SLC6A4) contains 14 exons and spans over 31 kb. Of special interest is the observation that at least two major polymorphisms within the SLC6A4 exist [26,27]. These are variable-number of tandem repeats (VNTR) polymorphism and both act as transcriptional regulators of the protein. One is positioned in the promoter region (5-HTTLPR) and is fixed in a short allele and a long allele, where the short allele is transcriptionally less active and links with vulnerability to affective disorders [25,35,82] and different clinical effect of selective serotonin reuptake inhibitors [83,84]. The second VNTR (VNTR-2) is located in the second intron of SCL6A4 and the alleles most frequently contain 10 and 12 tandem repeats where the 12 copy allele appears to support the 5-HTT expression distinct from that of the 10 copy allele [85,86]. However, also nucleotide elements of VNTR sequence may influence the efficiency of the transcription [85]. Although studies link the VNTR-2 to the number of platelets serotonin transporters [87], depression [88] and schizophrenia [89], there are disputes about the potential role of this genetic variant on human behavior [90].

Tourette's syndrome

Tourette's syndrome (TS) is a chronic neurological disorder with childhood onset. It is characterized by tics that wax and wane in severity and an spectrum of behavioral problems including attention deficit hyperactivity disorder (ADHD) and some forms of obsessive-compulsive disorder (OCD) [91]. The range of symptoms includes motor and phonic tics. Tics are defined as sudden repetitive movements, gestures or utterances that typically occur in bouts and look like aspects of normal behavior. Motor tics vary from sudden movements such as rapid forceful eye blinking, rapid head jerks and shoulder shrugs, to more complex or dystonic behaviors such as gestures of the hands, face and continuous head tilt [92].

Phonic or vocal tics spreads from simple sniffing or throat clearing to more complex words and phrases. Appreciated are also the sensorimotor phenomena that frequently accompany tics and obsessive-compulsive behavior. These experiences consist of premonitory feelings or urges that are relieved with the performance of the tic, and the patient experiences a momentary "just right" feeling [93]. Previous scientific efforts have not yet fully established the factors that influence the pathophysiology of TS. However, the natural history is likely to include gender specific factors, genetic, undesirable prenatal events, post-infectious autoimmune reactions, post-natal emotional stress and drug exposures, as well as comorbid medical and psychiatric conditions [94].

Many TS patients suffer from other comorbid psychiatric disorders including depression and various anxiety disorders [95]. TS was once thought to be a rare condition, the prevalence of TS is now estimated to be between 1 and 6 cases per 1000 boys, and milder variants of the syndrome are likely to occur in 1 to 10 percent of the population. The frequency of TS among adults is reduced by at least 75 % [96].

Table 2

Year	Author	N	♂ prevalence (%)
1973	Abuzzahab et al. [97]	173 cases in the USA 53 in the UK	
1988	Caine et al. [98]	142636	0.03
1990	Comings et al. [99]	3024	10*
1993	Apter et al. [100]	28037	0.04
1996	Robertson et al. [101]	166	2.9
1996	Costello et al. [102]	4500	0.1
2000	Kadesjo et al. [103]	435	0.15 - 1.0
2001	Hornsey et al. [104]	918	0.76

Epidemiology of Tourette's syndrome in the last three decades. A diverse range of ascertainment and evaluations were used, e.g. advertisement in news media, referrals from schools system or parents and screening system applied in school system of a county. Evaluation was made by self-report questionnaire and different structured interview by study physicians.

What is more, twin studies indicate that genetic factors are likely to play an important role in the transmission and expression of TS together with related phenotypes. Specifically, monozygotic twin pairs are highly concordant for TS (53 %) [105] . In addition, advances in molecular neurobiology have fostered efforts to examine the possible role of specific candidate genes in TS. Although numerous candidate genes, mostly involved in central monoaminergic pathways are investigated, there is limited evidence to support the role of some of these loci as genetic effects on TS. Having said that, a recent study shows promising progress in genetic research. The researchers found an association to a variation in a candidate gene (*SLITRK1*) which appears to enhance denditric growth in the wild-type [106].

Table 3

Tourette's syndrome characteristics
Emerge in childhood
Premonitory feelings
Temporary suppressibility
Increase with stress
Decrease with distraction and concentration
Waxing and waning, transient remissions
Boy to girl ration 4:1
No relationships to lifespan
No relationships to psychosis
OCD and ADHD are often comorbid disorders
Probably quite common – 1 % of children

Tourette's syndrome is a neurological disorder with onset in childhood. It is characterized by the presence of multiple motor tics and at least one phonetic tic. The table highlights important characteristics of the disease [96].

History of Tourette's syndrome

In 1825 the French neurologist Itard described a noble woman with body tics, barking sounds and uncontrolled utterances of obscenities. Sixty years later Gilles de la Tourette includes this woman in the descriptions of nine people. He notes that all nine patients share the feature of having brief involuntary movements or tics, six made noises, five were shouting obscenities, five repeated the words of others and two mimicked other people's gestures. Until the early 1960's, the disease was believed to be psychogenic, then the beneficial effects of antipsychotic drugs which antagonize the dopamine system were recognized and therefore refocused the etiology of the disease to a primarily organic disease involving a dysfunction of the CNS [96].

Neuroanatomical and neurochemical substrate in Tourette's syndrome

There is an extensive body of data that implicates the basal ganglia and related cortical and thalamic structures in the pathobiology of TS and related disorders. These data include a modest collection of neuropathological and neurosurgical studies as well as a fast growing literature based on *in vivo* neuroimaging techniques [107]. Functionally, the basal ganglia that contribute to the multiple parallel corticostriato-thalamocortical (CSTC) circuits serve a variety of sensorimotor, motor, oculomotor, cognitive and "limbic" processes [108,109]. For example, a functional magnetic resonance imaging (MRI) study of tic suppression indicates that deficits in reducing basal ganglia activation or difficulties in activating key prefrontal corticostriatal inhibitory pathways may be associated with increased tic severity [110].

The monoaminergic (dopaminergic, noradrenergic and serotonergic) projections that adjust the activity of the CSTC circuits have received large attention in connection with TS and in related conditions such as OCD and ADHD [91]. Despite their central role in the functioning of the CSTC circuits, the data concerning its roles remain sporadic [111]. However, support for the claim that the dopaminergic system is involved includes clinical trials in which haloperidol and pimozide, which preferentially block dopaminergic D_2 receptors, have been found to be effective in partial suppression of tics in a majority of TS patients [112,113].

The hypothesis for the involvement of 5-HT in motor activity has been present for some decades [114,115]. The most compelling evidence relates to the close connection to OCD and is based mainly on the well-established efficacy of serotonin reuptake inhibitors in the treatment of OCD [116]. However, these drugs prove to have restricted effectiveness in suppressing tics [117]. Of particular relevance, a study of 1400 Tourette's syndrome patients shows decreased plasma levels of the 5-HT precursor tryptophan [118] and postmortem studies in TS shows that 5-HT and the related compounds tryptophan and 5-hydroxy-indoleacetic acid (5-HIAA) may be globally decreased in the basal ganglia and other areas receiving projections from the dorsal raphe [119]. These findings are consistent with previous observations of significantly lower levels of CSF 5-HIAA [120], plasma TRP [121] and platelet 5-HT [118] in TS patients compared to normal controls, but not others [121,122]. Two *in vivo* studies investigated the 5-HTT density in drug free TS patients with the [^{123}I]β-cit SPECT tracer. The first found coherence between phonetic tic and reduced availability of 5-HTT [123]. The other clearly found reduced 5-HTT binding in TS patients [124].

In particular, the 5-HT_{2A} receptor is likely to be involved in motor control since 5-HT_{2A} receptor agonist treatment in rodents induces hyperlocomotion [125] and dopamine release in striatum [126]. Further, motor behavior and hyperlocomotion is attenuated by 5-HT_{2A} receptor antagonism [127,128]. In patients with Tourette's syndrome, risperidone, an atypical antipsychotic drug with affinity for the 5-HT_{2A} receptor is an effective treatment of tics [29] and ziprasidone and olanzapine, also atypical antipsychotics with high affinity for the 5-HT_{2A} receptor, have also showed promising treatment effects in case reports [129,130]. In addition, a small open clinical study has indicated that ketanserin, a compound with mainly 5-HT_{2A} antagonist properties, is effective for treating tics [131].

Aims

Taken together, the wide variety of observations showing that 5-HT_{2A} receptors can modulate cortical GABAergic transmission as well as glutaminergic and dopaminergic interactions indicates that a fine balance of 5-HT_{2A} receptor activation is required for a normal neuronal functioning in the cortex [132]. Increasing evidence supports that small changes in the genotype of 5-HT receptors and 5-HTT can modulate a number of developmental events. Consequently, these life-long changes in the 5-HT system could be an important factor in neurological and psychiatric disease since genetic variations in 5-HTT probably contribute to the risk of developing anxiety and mood related disorders [25,74] and have effect on antidepressants treatment response [133]. Even that our understanding of the precise response of GPCR signaling is limited, we believe that measures of the in *vivo* 5-HT_{2A} receptor constitute an important endophenotype to understand changes in the serotonergic system in health and disease.

Here, this thesis investigates the *in vivo* human brain distribution of radiolabeled altanserin binding to the 5-HT_{2A} receptor with PET in a population of healthy controls and patients with Tourette's syndrome.

The specific aims were:

i. To test the reproducibility of relevant outcome measures for 5-HT_{2A} receptor binding based on the bolus infusion design with [^{18}F]altanserin–PET. Here we evaluate the test-retest variability in six healthy subjects where regions of interest (ROI's) were automatically delineated and subsequently partial volume corrected. In addition, we also investigate the reproducibility of the accompanying values for non-specific binding, metabolite profile and plasma free radiotracer fraction (f_1).

ii. To calculate the required sample sizes to be used in clinically PET investigations to ensure enough power in future [^{18}F]altanserin PET bolus infusion research studies. A sample of 84 volunteers and adherent explaining variables constituted the basis for these power analyses.

iii. To test if genetic variations in the 5-HT_{2A} receptor and 5-HTT influence [^{18}F]altanserin binding to the 5-HT_{2A} receptor. We investigate the role of three single nucleotide substitutions in the 5-HT_{2A} receptor gene (-1438 *a/g*, 102 *c/t* and 452 *His/Tyr)* and two variable number of tandem repeats in the 5-HTT gene (5-HTTLPR and VNTR-2).

iv. To evaluate the 5-HT_{2A} receptor in Tourette's syndrome patients and healthy controls. Twenty patients with Tourette's syndrome and age- and gender-matched healthy control subjects were scanned to investigate regions to be particularly involved in Tourette's syndrome.

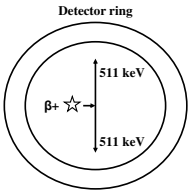
Material and methods

Positron emission tomography

Principle

PET is a non-invasive technique to study the distribution of tracer molecules labeled with positron emitting isotopes in the body and brain. The data obtained by detection of tracer molecules is used to reconstruct images describing the distribution of the tracer in the body and brain.

Figure 3



Physical principles of PET. The emitted positron (β+) from the radiotracer travels a short distance before annihilating with an electron (e-). The annihilating process forms two γ-photons (511 keV each) which spread in nearly opposite directions. These two are detected with a pair of opposed γ-ray detectors. Here displayed as a detector ring. To ensure that the detected γ-rays originate from the same annihilation only those signals are accepted which are detected within a short timing window. This technique is called coincidence detection.

At the time of radioactive decay, the positron has a relatively high energy as it travels through the brain tissue, Here, it interacts with electrons along the way, loses energy with each interaction before it annihilate. The degree of non-collinearity and the positron range sets the lower spatial resolution to about 3 mm. The systems used for detection of annihilation radiation are based on scintillation detectors such as bismuth germanate crystals coupled by a coincidence detection circuit. The accuracy of the image data are largely determined by sensitivity and spatial resolution of the PET camera.

Partial volume effect

The limited spatial resolution of PET influences quantitative PET measurements because many gray matter structures are small. The general thickness of cortical layers is between 3–5 mm. There is a cross-talk between gray matter, white matter and cerebrospinal fluid which influences the true measures of the tracer binding to the receptor. The direct consequence is that activities from surrounding compartments contribute to the signal measured in a region of interest (ROI), i.e. activity in a region is contaminated by activity from a neighboring region or from activity within a region (spill-over effect; partial volume effect (PVE)). These phenomena distort accurate quantification of radiotracer binding. Hence, several approaches have been evaluated for PVE correction to optimize the specificity of the quantitative measures in PET research [134].

PET imaging 5-HT_{2A} receptors in the brain

We refer to the Tourette's syndrome paper manuscript for detailed information of the PET method (appendices).

[^{18}F]altanserin is one of the most selective 5-HT_{2A} antagonists radiotracer and it has good reproducibility [41]. Radiolabeled metabolites from the tracer are found in plasma and cross the BBB and contribute to the total amount of radioactivity, but do not bind to 5-HT receptors [135]. Steady state conditions can be achieved within two hours by bolus infusion paradigm [40]. Two previous studies have demonstrated good agreement between [^{18}F]altanserin-PET and the distribution of 5-HT_{2A} receptors in human brain as determined by autoradiography [136] and decrease of [^{18}F]altanserin binding to the 5-HT_{2A} receptor when intervened with the 5-HT_{2A} receptor antagonist ketanserin [40]. These results confirm the high specificity of the radiotracer.

PET data acquisition and image analysis procedures have been described in detail previously [40,137,138]. In short, [^{18}F]altanserin is administrated as a combination of a bolus injection and a continuous infusion (ratio 1.75 hrs) to obtain tracer steady state in blood and tissue. Subjects receive a maximum dose of 3.7 MBq/kg bodyweight [^{18}F]altanserin. PET-scans are performed with an eighteen-ring GE-Advance scanner (General Electric, Milwaukee, Wisconsin, USA). MRI images is co-registered using a Matlab-based (Mathworks Inc., Natick, MA, USA) interactive program which is based on visual identification of the transformation [137]. MRI's is segmented into gray matter, white matter and cerebrospinal fluid tissue classes using overlay of prior probability images (SPM2). A total of 35 regions of interest (ROI's) is automatically delineated on MRI slices and transferred to PET images using the identified rigid body transformation [138]. PET images are PVE corrected using the segmented MRI. A three-compartment model based on gray matter, white matter and cerebrospinal fluid tissue is used [139,140]. The white matter value is extracted as the average voxel value from a white matter ROI (midbrain) in the uncorrected PET image.

Visual inspection of the time-activity curves is done to assure constant blood and tissue levels. The binding potential (BP_1) is calculated for cortical, limbic and subcortical ROI's. In a PET study it is not possible to measure the radiotracers affinity (K_d) and the concentration of receptor (B_{max}) directly. However, the volume of distribution (V_d) and

binding potential (BP) are highly related. V_d is defined as the ratio of radiotracer concentration in tissue ($c_{ROI\ and}\ c_{cerebellum}$) and plasma ($c_{plasma}$) at true equilibrium.

$V_{d(ROI)} = c_{ROI}/c_{plasma}$
$V_{d(NS)} = c_{cerebellum}/c_{plasma}$
$BP_1 = [V_{d(ROI)} - V_{d(NS)}]$

Where $V_{d(ROI)}$ and $V_{d(NS)}$ is volume of distribution of specific binding and non specific binding, respectively. In PET studies involving only one scanning it is usually assumed that radiotracer affinity is the same between groups and that any differences in radiotracer binding reflect differences in B_{max}.

Paper I: Reproducibility and sample size estimations of 5-HT_{2A} receptor measurements with [^{18}F]altanserin PET bolus plus infusion approach

To investigate the reproducibility of the method, we investigated six healthy Caucasian men (aged between 33 and 67 years) twice. Each person was MRI scanned once and PET scanned twice at an interval of two to fourteen days. In addition, to generate means and standard deviations of regional binding potentials for power calculations and sample size estimations, data from a large group of healthy controls (N = 84, 51 men, aged 18-74 years) were also included. This sample also included the first set of PET-images from the six subjects investigated in the test-retest study. Data on a part of this group (N = 52) have previously been reported by Adams et al. (2004) [141]. None of the subjects had a history of neurological or psychiatric disease and they all had a normal neurological examination on the day of PET-scanning. All subjects completed the Danish version of the 240 item NEO-PI-R self-report personality questionnaire on the same day as the PET scanning [142,143]. The volunteers were genotyped for the two length polymorphisms in the 5-HTT as described in detail in the next section.

Reproducibility

To examine the reproducibility of the prime outcome parameter, BP_1 and its components (non-specific binding, plasma concentrations, parent compound, and also f_1) differences were calculated as variability = absolute(test – retest) / ((test + retest)*0.5). To estimate the correlation between repeated measurements in the same subject, we calculated the intraclass correlation coefficient (ICC). ICC = ($MS_{Between}$ - MS_{within}) / ($MS_{Between}$ + (m-1)*MS_{within}) (statistical software package, SPSS) where m is the number of observations per subject, $MS_{Between}$ is the mean sum of squares between subjects and MS_{within} is the mean sum of squares within subjects. The variance of components was estimated from one-way analysis of variance.

Sample size estimation

Sample size estimates were calculated from ROI's mean and SD values of BP_1 and were based on the sample size required to detect a difference of 20 percent between two independent groups and a paired design with a significance level (α) of 0.05 and a power (β) of 0.8 (SAS statistical software package SAS 9.0; SAS Institute Inc., Cary, NC, USA). The age effect on [^{18}F]altanserin binding was taken into account by using the SD of the residuals from a simple regression model: residual = BP_1 – (age * slope + intercept), where slope and intercept was calculated from the sample of 84 subjects. The same procedure was used when adjusting for age, neuroticism and genetic covariates in same model; although a multiple regression model was used to generate the residuals. The describe analyses were execute on both PVE corrected data and not PVE corrected data.

Paper II: A serotonin transporter polymorphisms influences *in vivo* 5-HT_{2A} binding in the human brain

To investigate the influence of polymorphisms on the [^{18}F]altanserin binding in the brain, we examined 96 healthy individuals (36 women and 60 men) recruited via newspaper advertising. The median age was 42 years, age range was 18 to 80 years. All participants were screened and had no history of neurological or psychiatric disease. Likewise, they had no alcohol or substance history. On the day of PET scanning, they underwent a physical examination by a trained physician. All subjects completed the Danish version of the 240 item NEO-PI-R self-report personality questionnaire on the same day as the PET scanning [142,143].

Genotyping procedures

A literature search localized three regions with SNP's in the 5-HT_{2A} receptor gene (HTR2A; 13q14-q14.3) [144-146] and two VNTR's in the 5-HTT gene (SLC6A4; 17q11.1-q12) [35,147].

Genomic DNA was extracted from full blood and buffy coat lymphocytes using a purification set from Qiagen Incorporate (www.qiagen.com). The HTR2A -1438 *a/g* promoter polymorphism was identified by using the PCR protocol described by Masellis et al. (1998) [148]. 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'-ATG GCC TTT TGT GCA GAT TC-3' and followed by restriction enzyme digestion with *Msp*I for 4 h. PCR fragment were separated on a 2 % agarose gel (SeaKem GTG Agarose, www.cambrex.com). An *a* at position -1438 lead to an uncut fragment of 200 bp and a *g* at position -1438 lead to two fragments of length 121 bp and 79 bp.

The HTR2A 102 *t/c* polymorphism was identified using a PCR protocol described by Quist et al. (2000) [149]. In short, PCR amplification of a 242 bp fragment was generated by forward primer 5'-TCT GCT ACA AGT TCT GGC TT-3' and reverse primer 5'-CTG CAG CTT TTT CTC TAG GG-3' and followed by restriction enzyme digestion with *Msp*I overnight. PCR fragment were separated on a 2 % agarose gel

(SeaKem GTG Agarose, www.cambrex.com). A *t* at position 102 lead to an uncut fragment of 342 base pairs and a *c* at position 102 lead to two fragments of length 216 bp and 132 bp.

The 5-HT_{2A} receptor 452 *His*/*Tyr* polymorphism was identified using a PCR protocol described by Quist et al. (2000) [149]. In short, PCR amplification of a 155 bp fragment was generated by forward primer 5'-TGA TCG TTG GTT CCA CTA GAC TT-3' and reverse primer 5'-ATA CCG GCT TTG GGC CTA CA-3' and followed by restriction enzyme digestion with *Bsm*I for 4 hours. PCR fragment were separated on a 2 % agarose gel (SeaKem GTG Agarose, www.cambrex.com).The presence of the common *His* allele yielded two fragments of 95 bp and 60 bp while the *Tyr* allele produced one fragment of 155 bp.

The polymorphic VNTR (5-HTTLPR) upstream of the coding region of the 5-HTT gene was identified as described by Mellerup et al. (2001) [87]. In short, PCR amplification of a DNA fragment containing the polymorphic region was generated from the forward primer 5'-GGC GTT GCC GCT CTG AAT TGC-3' and reverse primer 5'-GAG GGA CTG AGC TGG ACA ACC AC-3' and followed by separation on a 2 % agarose (SeaKem GTG Agarose, www.cambrex.com). The presence of the short allele containing 6 copies of the tandem repeat yielded a fragment of 484 bp. The long allele containing 8 tandem repeats produced a fragment of 528 bp.

The VNTR element in intron 2 (VNTR-2) was identified using a PCR protocol described by Mellerup et al. (2001) [87]. In short, PCR amplification of a DNA fragment containing the polymorphic region was generated by forward primer 5'-TGT TCC TAG TCT TAC GCC AGT G-3' and reverse primer 5'-GTC AGT ATC ACA GGC TGC GAG-3' followed by separation on a 3 % agarose gel (NuSieve GTG Agarose, www.cambrex.com) yielded identification of 9, 10 and 12 copies of the tandem repeats.

Subjects were dichotomized as 5-HTTLPR (*s*/*s* + *s*/*l* vs. *l*/*l*) and VNTR-2 (*10*/*10* + *10*/*12* vs. *12*/*12*) consistent with previous studies demonstrating lower transcriptional efficiency of the promoter in short form (s) [150] and indications of 10 copies of VNTR-2 has lower expression than 12 copies [85,86,151]. The polymorphism in the HTR2A gene were not dichotomized (102 *t*/*c*: *t*/*t*, *t*/*c*, *c*/*c*; 452 *His*/*Tyr*: *His*/*His*, *His*/*Tyr*, *Tyr*/*Tyr* and -1438 *a*/*g*: *a*/*a*, *a*/*g*, *g*/*g*).

We applied multivariate repeated measures ANOVA (SAS statistical software package SAS 9.0; SAS Institute Inc., Cary, NC, USA) to analyze the effect of the polymorphisms in the HTR2A and SLC6A4 on 5-HT_{2A} receptor binding with [^{18}F]altanserin. Within-subject factors were 16 brain regions. Between-subject factor were genotype, age, and neuroticism.

Paper III: Cerebral 5-HT_{2A} receptor binding is increased in patients with Tourette's syndrome

Patients with a diagnosis of Tourette's syndrome (eight women and 12 men) were recruited from the Tourette's Syndrome Clinic at the Copenhagen University Hospital. Median age was 26 years; the age range was 17 to 49 years. The diagnosis was established by an experienced neurologist, based on Diagnostic and Statistical Manual of Mental Disorder, 4^{th} edition. Only patients with clearly visible tics and Tourette's syndrome as their primary disorder were included. Patients with intrusive obsessive or compulsive symptoms were excluded. Likewise, patients with mood disorders and other psychiatric disorders, including anxiety, were also excluded. All subjects completed the Danish version of the 240 item NEO-PI-R self-report personality questionnaire on the same day as the PET scanning [142,143]. Further, all subjects were genotype for the two 5-HTT length polymorphism described in the previous section.

Study of Tourette's syndrome patients

A general linear model was used to analyze difference in binding potential between Tourette's syndrome patients and healthy volunteers to ensure adjustment for the 5-HT_{2A} receptor decline with increasing age and a possible effect of neuroticism. In this model, were brain-region dependent variables and condition (Tourette's syndrome vs. healthy controls) age, and neuroticism independent variables (SAS statistical software package SAS 9.0; SAS Institute Inc., Cary, NC, USA). In addition, we performed a test for a general difference of BP_1 between controls and patients. Regional means were averaged across subjects for each condition and we used PROC GLM with age and condition as covariates as described above. However, this relies on the normal distribution assumption, so for robustness considerations, we performed a randomization test (100000 random group allocations) to evaluate the test statistic for condition [152].

Results and discussion

Reproducibility of 5-HT_{2A} receptor measurements with [^{18}F]altanserin PET bolus plus infusion approach

The spatial resolution and statistical reliability in PET is expectedly lower than measurements from brain tissue samples and besides, *in vitro* and *in vivo* binding data most often differ. Therefore, binding parameters obtained from postmortem human brain studies may not be directly comparable to in human *in vivo* imaging data. Knowledge of the intraindividual variability as determined by repeated PET measurements would be useful for the validity and design of current and future PET-studies. A measure may be reliable but not valid, but it cannot be valid without being reliable. In paper I, we evaluate the binding potential (BP_1) in brain regions with PET-[^{18}F]altanserin by scanning the same person twice with an interval of 2 to 14 days. Between-subject variability and ICC is used to measure test-retest reliability. ICC may be conceptualized as the ratio of between-groups variance to total variance. With a small sample size (<15), ICC is preferred over Pearson's correlation coefficient.

We found that reproducibility of [^{18}F]altanserin binding in six males supports the feasibility of the [^{18}F]altanserin bolus plus infusion approach for determination of 5-HT_{2A} receptor binding in humans. In contrast to previous studies addressing the same aim, this investigation implicates automatic region of interest delineation and partial volume correction together with analyses of single regions in one hemisphere. In regions with high tracer binding the outcome parameter (BP_1) showed high reproducibility. ICC's were in the range of 0.67 to 0.93 where maximum is 1.0 and the variability between test and retest was 5 % to 12 %. In regions with low receptor density, BP_1 reproducibility was lower. The results are highlighted in Table 4.

In general, the variability found in cortical regions is slightly lower than those reported in a [^{18}F]altanserin test-retest by Smith et al. [41]. They assessed the distribution volume by a four-compartment model and the Logan graphical method. In contrast to our study, they did not execute PVE correction, which we found to reduce the total variability in a big population of healthy volunteers (see next section). Their choice of regions was large pooled cortical regions covering both hemispheres, which on the other hand should reduce the variability between test and retest.

In conclusion, the current study supports the feasibility of the [^{18}F]altanserin bolus plus infusion approach combined with a user independent delineation of regions of interest and PVE correction for determination of 5-HT_{2A} receptor binding in humans. Obviously, the low receptor density regions were very sensitive to differences in coregistration, segmentation, and automatic delineation and PVE correction. In these regions, there are too little signal and too much noise and the gray matter structures have only an extent of few millimeters in each slice.

Sample size estimation of 5-HT_{2A} receptor measurements with [^{18}F]altanserin PET bolus plus infusion approach

How many subjects should be included in a PET study to reach a valid conclusion? This is a question frequently asked by clinical investigators. By performing sample size estimation, we estimate how many persons are needed in the PET design to optimize precision to provide reliable answers. Without these calculations, the sample size may be too high or low. The power (β) is defined as the probability of detecting a true effect. Alpha (α) is the probability of detecting a false effect.

The sample size issue is particularly important if a study demonstrates no statistically significant difference, because the first interpretation is that the results of the statistical test are correct and that there truly is no statistically significant difference. The second interpretation is that the results of the statistical test are erroneous and that there is an underlying difference, but the study is not powerful enough (sensitive enough) to detect it and therefore yielding a false-negative result. In statistical terminology, a false-negative result is known as a *type II error*. An adequate sample size gives a statistical test enough power so the first interpretation is much more plausible than the second interpretation.

It is our impression that many published clinical PET studies possess low statistical power owing to inadequate sample size. An appropriate sample size generally depends on five study parameters: minimum expected difference (miredif), estimated or calculated measurement variability, desired statistical power, significance criterion and whether the analysis is one-tailed or two-tailed. In this study, we set the minimum expected difference at 20 %. The setting of this parameter is only a guideline. If miredif is smaller, the sample size needed to detect statistical significance increases. The estimated measurement variability was determined on the basis of 84 PET scanned healthy subjects and adjusted for age, and analyses were done before and after PVE correction. As statistical variability increases, the sample size needed to detect the minimum difference also increases. A high power is always desirable, but there is an obvious trade-off between the number of individuals it is feasible to study and the amount of time and resources available. The power and significance values were set to 0.8 and 0.05, which are commonly used parameters.

When adjusting for the genotypes of VNTR's in 5-HTT gene (paper II) and neuroticsm [142], we succeed in decreasing the estimated sample size further (Table 4). The sample sizes for paired design were, as expected, much lower. We did not find any gender effect on [^{18}F]altanserin binding (F=1.91, P=0.17). Genotypes of polymorphism in the 5-HTT and neuroticism are newly discovered covariates to the [^{18}F]altanserin binding and are not published in paper I.

Table 4 shows that controlling for parameters that influence binding gives a more sensitive method. In conclusion, there is a decline in sample sizes after PVE correction in cortical regions of an average of 86 %. This implies that technical aspects of PET are important to master to gain effect of the method capability. Furthermore, by adjusting for genotype and neuroticism further reduces the sample size an average of 12 %.

Table 4

		test (N = 6)			retest (N = 6)			Variabilty (%)			Sample size (N = 84)					Sample size (N = 84)		
Data	Region (left hemiphere)	Median	mean	SD	Median	Mean	SD	Median (95% CI)	PVE ICC	Non PVE ICC	Mean	PVE SD	*Na*	*Nb*	*Nc*	Mean	Non PVE SD	*N*
Cp		3.90	4.09	0.58	4.01	4.14	0.50	7 (4 - 10)	0.79		3.12	1.06				3.12	1.06	
alt_frac		52.44	52.51	8.71	53.12	53.37	5.43	9 (4 - 14)	0.71		51.85	7.71				51.85	7.71	
fl		0.32	0.34	0.10	0.33	0.33	0.08	25 (18 - 32)	0.57		0.42	0.22				0.42	0.22	
Cp_cor		2.11	2.15	0.50	2.29	2.21	0.33	11 (5 - 17)	0.80		1.66	0.62	Two sample	Paired	Two sample	1.66	0.62	Two sample
Vd	cerebellum	2.06	2.09	0.69	2.10	2.14	0.61	12 (5- 17)	0.92	0.90	2.11	0.66				1.76	0.59	
BP1	*High density regions*																	
	orbitofrontal c.	2.12	2.11	0.37	2.19	2.16	0.39	7 (5 - 9)	0.91	0.90	2.32	0.60	28	16	23	1.30	0.44	46
	med. inf. frontal c.	3.08	2.85	0.70	2.73	2.72	0.56	5 (0 - 10)	0.90	0.93	2.93	0.71	24	14	22	1.31	0.42	42
	sup. frontal c.	2.94	2.85	0.66	2.68	2.81	0.60	6 (2 - 10)	0.92	0.94	2.94	0.71	24	14	22	1.22	0.39	43
	ant. cingulate	2.28	0.62	0.62	2.00	1.93	0.54	12 (2 -21)	0.79	0.86	2.12	0.58	30	17	28	1.39	0.46	54
	post. cingulate	2.20	2.19	0.42	2.30	2.22	0.34	6 (2 - 10)	0.90	0.94	2.36	0.60	27	15	22	1.50	0.46	38
	insula	1.95	1.93	0.50	1.83	1.82	0.44	6 (0 - 12)	0.89	0.93	1.92	0.51	28	16	25	1.43	0.46	44
	med. inf. temporal c.	2.31	2.20	2.20	2.20	2.15	0.49	9 (0 - 21)	0.67	0.78	2.43	0.61	27	15	24	1.41	0.45	42
	sup. temp c.	2.43	2.35	0.56	2.45	2.36	0.49	5 (0 - 11)	0.89	0.94	2.56	0.66	28	16	24	1.43	0.46	41
	sens. motor c.	3.03	2.77	0.62	2.93	2.80	0.64	6 (2 - 10)	0.93	0.94	2.72	0.70	27	15	24	0.96	0.35	55
	parietal	3.26	3.02	0.65	2.96	3.03	0.62	6 (1 - 11)	0.91	0.93	3.18	0.76	24	14	20	1.27	0.44	50
	occipital c.	3.01	2.74	0.66	2.88	2.74	0.61	5 (0 - 10)	0.93	0.93	2.85	0.72	26	15	23	1.34	0.43	41
	Low density regions																	
	entorhinal c.	0.97	0.88	0.31	0.75	0.72	0.37	39 0 - 80)	0.02	0.73	0.91	0.44	86	45	82	0.36	0.31	309
	hippocampus	0.51	0.46	0.14	0.51	0.47	0.23	27 (0 - 60)	0.38	0.51	0.51	0.24	92	48	85	0.45	0.24	111
	thalamus	0.81	0.24	0.24	0.79	0.74	0.11	11 (0 - 22)	0.44	0.40	0.66	0.25	60	17	60	0.31	0.18	143
	caudate	0.89	0.89	0.28	0.69	0.80	0.20	17 (0 - 34)	0.38	0.56	0.68	0.30	74	39	60	0.22	0.18	205
	putamen	0.83	0.85	0.19	0.80	0.81	0.06	17 (7 - 27)	0.14	0.10	0.73	0.27	52	28	45	0.54	0.20	64

Variabilities, intraclass correlations and estimations of sample sizes.
Parametric measures of volume of distribution (V_d) of cerebellum and regional binding potentials (BP_1) together with variability between test and retest and the intraclass correlation (ICC). For each region, estimated sample sizes are shown to detect changes with sufficient power between two independent groups and in paired design. Abbreviations: PVE = partial volume estimation, Cp = plasma concentration (non corrected), alt_frac = [^{18}F]altanserin fraction, f_1 = free fraction of [^{18}F]altanserin, Cp_cor = metabolite corrected plasma concentration, cb = cerebellum. Further, data from 84 subjects (adjusted for age) were used for calculation of distribution and expected sample size to detect regional changes between two independent groups (*Na*) and in paired design (*Nb*). Data *Nc* were adjusted for age, neuroticism, 5-HTTLPR and VNTR-2 (VNTR in second intron of 5-HTT).

As for the reproducibility study, sample size estimations show the same pattern, e.g. that cortical and limbic regions show reasonable sample size to ensure enough power, whereas the smaller regions with low density need a big sample size. The results reported were conducted with a fixed power (0.8) and a minimum expected difference set at 20 %. As a consequence of increased power and minimum expected power set at, for example, 10 percent, the sample size will increase. Thus, altanserin-PET is less suitable to detect subtle differences in low-density regions, because this would require prohibitively large samples. However, a paired (intervention) design will probably have a higher value because of the lower individual variability.

Besides, an adjustment for the variability induced by age, neuroticism scores, and polymorphism gain more homogenous results. From a methodological view future studies will benefit from these results by selection of the sample and stratifying for these effects. The results support feasibility of the [^{18}F]altanserin bolus plus infusion approach for determination of 5-HT_{2A} receptor binding in humans when looking at cortical and limbic regions.

Polymorphism and influence on 5-HT$_{2A}$ receptor binding

Results from the genotyping of the healthy Caucasian humans are presented in Table 5. Genotype frequencies of HTR2A (-1438 *a/g*, 102 *t/c* and 452 *His/Tyr*) and SLC6A4 gene polymorphisms (5-HTTLPR and VNTR-2) in the PET group of healthy volunteers all conformed to the Hardy-Weinberg equilibrium. The SNP site -1438 *g/a* and 102 *t/c* were in perfect disequilibrium so the genotypes were treated as one. The 5-HT$_{2A}$ receptor SNP at site 102 *t/c* and 452 *His/Tyr* showed no significant effect on [^{18}F]altanserin BP$_1$ values, neither in multivariate repeated measures analysis or single regional binding comparisons. Consequently, they were excluded in the further analysis to reduce noise.

Table 5

Polymorphism	Genotype	Distribution	Percentage	Hardy-Weinberg Chi-square (P value)
102 *t/c*	***t/t***	**14**	**14.6**	**0.83**
	t/c	**44**	**45.8**	
	c/c	**38**	**39.6**	
452 *h/y*	***His/His***	**81**	**84.4**	**0.41**
	His/Tyr	**15**	**15.6**	
	Tyr/Tyr	**0**	**0.0**	
5-HTTLPR	***s/s***	**19**	**19.8**	**0.29**
	s/l	**53**	**55.2**	
	l/l	**24**	**25.0**	
VNTR-2	***12/12***	**35**	**36.5**	**0.12**
	10/12	**36**	**37.5**	
	10/10	**24**	**25.0**	
	9/9	**0**	**0.0**	
	9/10	**0**	**0.0**	
	9/12	**1**	**1.0**	

Group characteristics and genotype frequencies for 5-HT$_{2A}$ and 5-HTT polymorphisms in the investigated population of healthy Caucasian volunteers. 102 t/c and 452 *His/Tyr* are polymorphisms in the 5-HT$_{2A}$ receptor gene. 5-HTTLPR and VNTR-2 are length polymorphisms in the 5-HTT gene.

Frokjaer et al. (submitted) [142] reports an association between increased [^{18}F]altanserin binding and increased neuroticism score in healthy volunteers. This personality dimension was assessed by the questionnaire, NEO-PI-R. Neuroticism score reflects individuals emotions such as tension, worries, guilt and sadness. In addition, as described in the previous section, we were able to decrease the variability by adjusting for the effect of neuroticism. In a regression model where neuroticism score were the dependent variable and the polymorphisms independent class variables we tested the group allelic effect of each polymorphism on neuroticism score (mean: 71, range: 34 - 118). Single and multiple regression results did not find any polymorphic effect on neuroticism score (Table 6).

Table 6

Dependent variable Neuroticism score	Independent variable Polymorphism	F value	P value
34 - 113	VNTR-2	3.46	0.07
	5-HTTLPR	0.03	0.85
	102 *t/c*	0.52	0.31
	452 *His/Tyr*	0.15	0.70

The single effect of each polymorphism on neuroticism score in 96 healthy individuals tested in a simple regression model. Inclusion of all independent variables in a multiple regression model did not explain the variation in neuroticism score either.

Multivariate repeated measures ANOVA of the group allelic effect of VNTR-2 on 5-HT_{2A} receptor binding were significant, F = 4.18, P = 0.04, both when adjusting for age and neuroticism (Table 7 and 8). We did not find any allelic effect of 5-HTTLPR on 5-HT_{2A} receptor binding, but adding the 5-HTTLPR as a covariate to the model the allelic effect of VNTR-2 got more significant (Table 9 and 10). Including neuroticism score to the model did only, however, influence the result to a minor extent. In general, BP_1 values of VNTR-2 *10/10* and *10/12* were universally lower in all tested brain regions than subjects carrying *12/12* (Fig. 4).

Table 7

Within subjects factor	Between subjects factor	F value	P value
Brainregions	Age	8.37	0.005
	VNTR-2	4.15	0.04

Multivariate repeated measures ANOVA. Effect of covariates on 5-HT_{2A} receptor binding with [^{18}F]altanserin. Within-subject factors were 16 brain regions (Fig. 4). Between-subject factor were VNTR-2 in the 5-HTT gene and age.

Table 8

Within subjects factor	Between subjects factor	F value	P value
Brainregions	Age	4.17	0.04
	Neuroticism	4.39	0.04
	VNTR-2	4.39	0.04

Multivariate repeated measures ANOVA. Effect of covariates on 5-HT_{2A} receptor binding with [^{18}F]altanserin. Within-subject factors were 16 brain regions (Fig. 4). Between-subject factors were VNTR-2 in the 5-HTT gene and age and neuroticism.

Table 9

Within subjects factor	Between subjects factor	F value	P value
Brainregions	Age	9.86	0.002
	VNTR-2	6.00	0.02
	5-HTTLPR	2.95	0.09

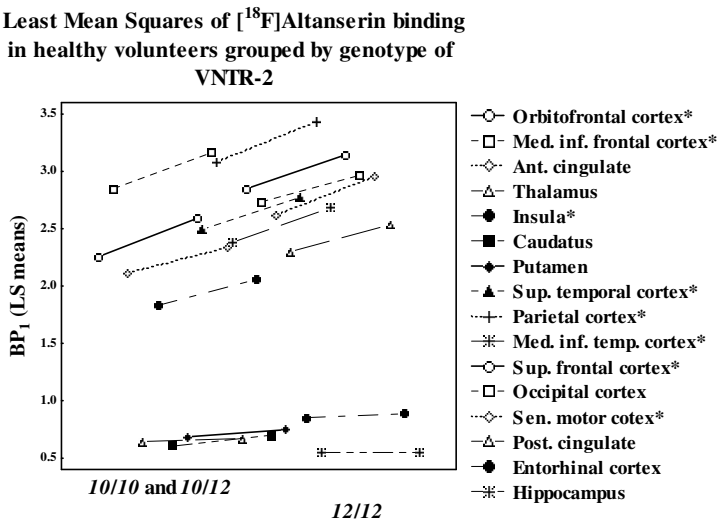
Multivariate repeated measures ANOVA. Effect of covariates on 5-HT_{2A} receptor binding with [^{18}F]altanserin. Within-subject factors were 16 brain regions (Fig. 4). . Between-subject factor were VNTR-2 and 5-HTTLPR in the 5-HTT gene and age.

Table 10

Within subjects factor	Between subjects factor	F value	P value
Brainregions	Age	5.14	0.03
	Neuroticism	4.63	0.03
	VNTR-2	6.23	0.01
	5-HTTLPR	2.83	0.10

Multivariate repeated measures ANOVA. Effect of covariates on 5-HT_{2A} receptor binding with [^{18}F]altanserin. Within-subject factors were 16 brain regions (Fig. 4). Between-subject factor were VNTR-2 and 5-HTTLPR in the 5-HTT gene and age and neuroticism.

Figure 4



Effect of serotonin transporter VNTR-2 genotype on *in vivo* 5-HT_{2A} receptor binding in healthy volunteers. A general linear model with regional 5-HT_{2A} receptor binding in brain regions as dependent variables and VNTR-2 polymorphism in the 5-HTT gene and age as independent variables was used. The

VNTR-2 polymorphism is dichotomized: *10/10 + 10/12* (left points) vs. *12/12* (right points). The figure displays adjusted regional 5-HT_{2A} binding means for the two groups. * (P<0.05) indicates significant group difference after *post hoc t* test.

Here we observe that a group allelic effect of VNTR-2 reduces 5-HT_{2A} receptor binding in *10/10* and 10*/12* individuals compared to 5-HT_{2A} receptor binding in *12/12* individuals. This effect was strengthened by adding the functional 5-HTTLPR to the model. These findings provide support for evolutionary mechanisms that affect the transcriptions to generate polymorph regulatory elements. Thereby, our hypothesis that differential expression of the 5-HTT in relation to 5-HTTLPR and VNTR-2 underlie regulation of serotonergic neurotransmission [85,150,153] and, as a consequence, the regulation of the distinct but functional connected 5-HT_{2A} receptor.

The influence by 5-HTTLPR, further argues that the transcriptional 5-HTTLPR element act in concert with regulatory elements in the second intron, as suggested in previous studies [88,154].

The 5-HTT protein works by sodium dependent reuptake of 5-HT into the presynaptic neuron, but the basis of this activity is poorly understood. We suggest that low expression of 5-HTT due to the *10* and *s* allele leads to lifelong increase in endogenous 5-HT tone and desensitization and down-regulating of 5-HT_{2A} receptors. Previous studies and scientific reviews support that regulations of the 5-HT_{2A} receptor may be a common mechanism to adjust serotonergic transmission in response to physiological demands [68,155]. Other *in vivo* imaging studies support our findings. One study reports higher 5-HTT availability in the raphe area in healthy volunteers homozygous for the long insertion *l/l* [156] which connects to higher transcription activity for the *l* allele. Another study shows that carriers of the *s* allele of 5-HTTLPR reduce 5-HT_{1A} receptor availability in humans which could be due to desensitization [157].

The neuroticism score did not associate to the functional length polymorphisms in the SLC6A4 and SNP's in HTR2A. However, it is our opinion that studied association between complex behavioral traits and polymorphisms are problematic in small singular studies. Nonetheless, numerous experimental and human studies links length polymorphisms in the 5-HTT gene to diseases [89] and behavioral traits [35], and, further, suggest that these genotypes can predict the risk of developing neuropsychiatric diseases [25]. However, it must be emphasized that the literature not consistently points towards an involvement of the SLC6A4 in neuropsychiatric diseases. For example, a metaanalysis by Schinka et al. (2004) [158] did not provide statistical strength for an influence of the short/long polymorphism on anxiety. Further, another metaanalysis did not find associations between the VNTR in intron 2 and affective disorders [159]. Also, an Australian study [160] did not replicate the findings of Caspi et al. (2003) [25].

Previous PET studies address differences in the density of the 5-HT_{2A} receptor in the brains of depressive patients [59,161,162] and schizophrenic patients [163], but other studies, however, do not show any difference in tracer binding in depressed [60,164,165] and schizophrenic patients [166,167]. Whether the functional length polymorphisms in our sample are linked to a behavioral state is at the moment unknown. Power problems and problems with multiple comparisons will probably make it statistically difficult to tell in a sample of this size.

As a consequence, we believe that the universal association between functional length polymorphisms and [^{18}F]altanserin binding is an important congenital personal trait. This observation corresponds nicely to the findings by Pinborg et al. (submitted) [168] who nicely address a striking association in [^{18}F]altanserin binding between monozygotic twins compared to dizygotic twins.

In contrast, we did not find any effect in [^{18}F]altanserin binding of the tested SNP's in the 5-HT_{2A} receptor gene (102 *t/c* and 452 *His/Tyr*). For 452 *His/Tyr* a low frequency of the *Tyr* allele makes it difficult to establish significant changes in [^{18}F]altanserin binding. For 102 *t/c* it is in accordance with a previous study showing no effect on 5-HT_{2A} mRNA levels in temporal post mortem tissue of normal subjects [169]. However, another study reports that healthy carriers of the *c* allele express 20 % lower mRNA than the *t* allele [170]. Broadly speaking, there is still no consensus in the literature since studies in healthy and sick people show inconsistent results assessing differences between 102 *t/c* genotypes at mRNA and protein level [78,171-174].

To sum up briefly, we question whether differences in the 102 *t/c* locus and 452 *His/Tyr* locus are associated with direct effects of protein expression or conformational alterations in the binding pocket of the 5-HT_{2A} receptor protein. However, it is theoretically possible that our findings will not be relevant to the developmental nervous system, as we investigated binding in only healthy adult brains, and that different polymorphisms may only influence the developing brain.

We believe that the observed widespread homogenous allelic effect of the VNTR-2 and trend effect of 5-HTTLPR refutes the claim that the results are incidental findings. The post-hoc analysis showed that BP_1 was lower by 5-40 % in cortical regions of the individuals carrying 10 repeat copies of the VNTR. Correspondingly, the largest difference was observed between individuals holding a short allele in the promoter of 5-HTT and the 10 repeat VNTR in second intron versus individuals homozygous for the 12 repeat copies in VNTR-2 and long allele in 5-HTTLPR (data not shown). By comparison, the test-retest variability of this measure was 5-12 % in cortical regions [175], thus, the observed changes appear to be robust findings.

All together, this is the first PET study that investigates the functional polymorphisms in the 5-HTT on the distinct but functionally connected 5-HT_{2A} receptor. This will affect future PET studies of the 5-HT_{2A} receptor because data have to be stratified according to the involved polymorphism. As shown in Table 4, this maneuver will reduce the variance and, as a consequence, a smaller sample may generate reliable answers in research investigations.

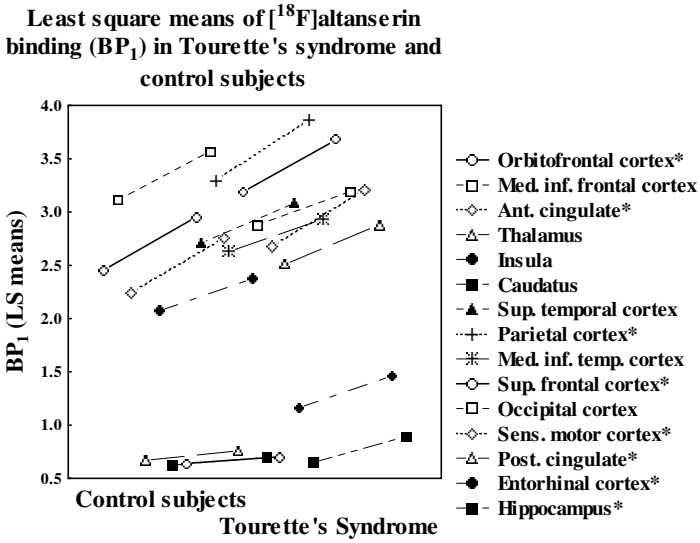
Furthermore, the results complement the expanding literature about the regulatory mechanisms in the 5-HT system with special reference to the 5-HTT and 5-HT_{2A} receptor. Considering the pivotal role of 5-HT in neurodevelopment and as a modulator in the nervous system, our results add relevant news about regulation of the 5-HT_{2A} receptor, genetic influence on the 5-HT system, genetic link to disease and genetic basis for differential drug response. On the other hand, we are well aware that the complex traits and behavior dimensions are most likely to be generated by broad interaction between environmental factors and a number of genes, regulatory proteins and their products. Though, careful interpretations should be done on behalf of these data.

PET imaging of Tourette's syndrome patients

In patients with Tourette's syndrome [^{18}F]altanserin binding was significantly increased in the cortical brain regions hypothesized to be involved in the disease (ant. cingulate, orbitofrontal cortex, and sup. frontal cortex) (Fig. 5). In addition, a post-hoc analysis identified a global difference between controls and patients with a higher BP_1 in Tourette's syndrome patients (P = 0.03). A separate analysis of the ten drug free patients also showed a statistically significant increased 5-HT_{2A} receptor binding as compared to their age-matched controls. There was no statistically significant difference in BP_1 between Tourette's syndrome patients with an YBOCS score under and over 10 or between drug-free and drug-treated patients. Further, a comparison of pimozide-treated TS patients (n = 7) with the rest of the patients (n = 13) and healthy control subjects (n = 20) was carried out and revealed no differences in terms of cerebellar uptake, plasma concentration, parent compound of tracer or binding potentials. In addition, the pimozide-treated patients had the same clinical scores as the rest of the patients. A regression analysis did not show any significant relations between age-adjusted BP_1 and tic severity or YBOCS score, neither when stratification for drug use was carried out. Regression analyses associating BP_1 with tic severity carried out for the combined and separate scores for motor and vocal tics, as well as for obsessions and compulsions did not reveal any significant associations.

Genotypes of polymorphism in the 5-HTT and neuroticism are newly discovered covariates to the [^{18}F]altanserin binding and are not published in paper III. In terms of the *s* allele of 5-HTTLPR and *12* allele of VNTR-2, controls and patients with Tourette's syndrome showed allele frequencies that were identical with their respective controls. It is not reasonable to expect major differences with the limited sample size. Neuroticism scores were significant different between controls, Mean: 79, SD: 18, and TS patients, Mean: 100, SD: 18 (students t-test, P = 0.001). However, we did not find any association between neuroticism scores and [^{18}F]altanserin binding in TS patients in a multivariate GLM analysis with age as a covariate (F = 0.00, P = 0.95). This variable was omitted in further analyses. In comparison the age effect was: F = 6.92, P = 0.02, and included in all analyses.

Figure 5



Age adjusted means of [^{18}F]altanserin binding in ROI's of healthy volunteers and Tourette's syndrome patients. These regions are average between left and right hemisphere. Results showed a global increase in 5-HT_{2A} receptor binding in TS patients. Left points are average regional binding in control subjects and right points are average regional binding in TS patients. *P <0.05 indicates significant group difference.

Our finding of a global increase in 5-HT_{2A} binding may result from conformational changes in the receptor associated with a higher affinity of the 5-HT_{2A} receptor. We would like to emphasize, however, that both an increased B_{max} and an increased 5-HT affinity could result in an augmented stimulation of the 5-HT_{2A} receptor. Such stimulation from frontal regions may activate the striosomes (either directly or via dopamine) and induce urges or motor tics [45,176]. This is supported by several studies in both humans and rodents where global agonist activation of the 5-HT_{2A} receptor with DOI ((+/-)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane), fenfluramine and psilocybin have been shown to increase synaptic dopamine levels in the brain striatum [126,177,178]. It has previously been reported that Tourette's syndrome patients have decreased D_2-receptor binding after challenge with amphetamine which is consistent with the notion that dopamine levels are increased in these patients[179]. Our data could suggest that increased striatal dopamine release in Tourette's syndrome patients is brought about by over-stimulation of 5-HT_{2A} receptors, but based on our data, we cannot conclude if the changes in 5-HT_{2A} receptor are primary or secondary to other alterations. For example, the increase of 5-HT_{2A} receptor binding could be a relevant compensatory response to Tourette's syndrome pathophysiology or it could be caused by a reduction in synaptic 5-HT levels. At present, we do not know how the *in vivo* [^{18}F]altanserin binding is affected by long-term changes of 5-HT levels, but acute increases in synaptic 5-HT do not affect the binding of [^{18}F]altanserin [180]. The reduced 5-HTT binding in Tourette's syndrome patients reported by Muller-Vahl et al.

(2005) [124] and Heinz et al. (1998) [123] can be speculated to be associated with changed 5-HT levels. However, whereas a primary decrease in 5-HT levels is likely to be associated with a reduction in SERT, a primary reduction in SERT is more likely to result in increased synaptic 5-HT levels. Some studies have, however, supported the notion of decreased cerebral 5-HT levels in Tourette's syndrome patients [119], but it is unclear whether this is causally related to the presence of tics, and further, results have been contradictory [122]. To date, there is no clinical evidence that SSRI treatment improves tic severity [181].

Conclusions and perspectives

In conclusion, ^{18}F]altanserin PET is a reliable method for *in vivo* 5-HT_{2A} receptor binding in the brain, and our data show that the method is sufficiently sensitive for the examination of the 5-HT_{2A} receptors especially when adjusting for age, VNTR-2 and 5-HTTLPR, and neuroticism. Even more importantly, PVE correction reduces the sample sizes considerably. In future [^{18}F]altanserin-PET studies, it is important to adjust for covariates that influence the binding potential, and perhaps even better, to make sure that the samples are as homogenous as possible. We believe that one of the reasons for the often conflicting outcome of clinical PET studies may be caused by insufficient power – most studies include no more than 10-15 subjects in each group. Our data underline the importance of carefully addressing the power issue. On the other hand, we are well aware of the trade off clinicians have to consider in clinical PET between time and cost. Also, one could argue that the power issue is particularly important to rule out type II errors, that is, when no differences are identified. We suggest following promising research projects:

- Conduction of a test-retest [^{18}F]altanserin-PET study spanning over a longer period will reveal long-term stability of the *in vivo* measure of 5-HT_{2A} receptor binding in humans.

- Continuously execute the [^{18}F]altanserin bolus-infusion method on healthy controls and collect careful and uniform, broad demographic information on the enrolled subjects. In the future, this could help identifying new covariates like e.g. body mass index, food preferences, gender, sleeping habits and new polymorphism. Further, implementation of the present results in previous published data could disclose new facts and thereby other perspectives of the serotonergic system.

Polymorphisms in the 5-HT_{2A} receptor gene have been associated with several neuropsychiatric disorders. In healthy individuals we did not, however, find any associations between frequent SNP's in the HTR2A (102 *t/c* and 452 *His/Tyr*) and *in vivo* brain 5-HT_{2A} receptor binding. By contrast, for the VNTR in the second intron of the 5-HTT gene and probably also the short and long allele in the promoter of the 5-HTT gene we found that these functional length polymorphisms were associated with differences in the 5-HT_{2A} receptor binding in the living human brain. These results confirm previous studies showing that the differential expression of the 5-HTT affects the distinct but functional related 5-HT_{2A} receptor. In this area, we would like to suggest the following promising future research projects:

- Evaluate the possible interplay between other polymorphisms involving the 5-HT system and the functional length polymorphisms in the 5-HTT gene on the *in vivo* 5-HT_{2A} receptor binding. For example, the brain-derived neurotrophic factor (BDNF) is supposed to interact with 5-HT during development.

- Search for a role of differential expression of 5-HTT on behavior. This could be done by carrying out behavioral studies on transgenic mice engineered to differential expression of 5-HTT according to genotypes.

- The estimated required sample size is lower for paired design. This represents a reason to focus on intervention studies to evaluate the physiological and pathophysiological mechanism in the serotonergic neurotransmission. For example the *in vivo* relation between long term drug response in relation to functional polymorphism in the 5-HTT gene.

- Compare [^{18}F]altanserin-PET and [^{11}C]DASB-PET in healthy volunteers to evaluate the correlations between the 5-HT_{2A} receptor and 5-HTT.

Our findings of increased [^{18}F]altanserin 5-HT_{2A} receptor binding in Tourette's syndrome patients indicate a role for the serotonergic system in the manifestation of tics, either directly via the striosomal compartment in striatum or through an increased dopaminergic tone. However, it was not possible to demonstrate a correlation between tics and OCD severity and regional 5-HT_{2A} receptor binding. This analysis was, however, probably hampered by a narrow variance in tic severity. To this field of research we suggest following promising future projects:

- Combination of our finding of an increase in 5-HT_{2A} receptor binding and the previous case-based observation that the 5-HT_{2A} antagonist ketanserin may ameliorate tics in patients with Tourette's syndrome [131] suggests that the effect of 5-HT_{2A} antagonist treatment should be investigated in a clinical trial.

- With the development of the highly specific PET-tracer [^{11}C]DASB for the 5-HTT [182] and the described reference–tissue method for data analyses [183] it is highly interesting to investigate the 5-HTT in conjunction with the 5-HT_{2A} receptor in the same group of Tourette's syndrome patients. First, this will add further to the role of 5-HT. Second, in pure TS patients it will eliminate the obsessive-compulsive component. Third, it will replicate similar studies with the less specific [^{123}I]β-cit SPECT-tracer.

References

[1] *Boguski MS, Jones AR. Neurogenomics: at the intersection of neurobiology and genome sciences. Nat Neurosci 2004;7:429-433.*

[2] *Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res 2005;33:D514-517.*

[3] *www.nih.gov.*

[4] *Olesen J, Leonardi M. The burden of brain diseases in Europe. Eur J Neurol 2003;10:471-477.*

[5] *Roth BL, Hanizavareh SM, Blum AE. Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. Psychopharmacology (Berl) 2004;174:17-24.*

[6] *Schloss P, Williams DC. The serotonin transporter: a primary target for antidepressant drugs. J Psychopharmacol 1998;12:115-121.*

[7] *Staley JK, Malison RT, Innis RB. Imaging of the serotonergic system: interactions of neuroanatomical and functional abnormalities of depression. Biol Psychiatry 1998;44:534-549.*

[8] *Popa D, Lena C, Fabre V, Prenat C, Gingrich J, Escourrou P, Hamon M, Adrien J. Contribution of 5-HT2 receptor subtypes to sleep-wakefulness and respiratory control, and functional adaptations in knock-out mice lacking 5-HT2A receptors. J Neurosci 2005;25:11231-11238.*

[9] *Frank GK, Kaye WH, Meltzer CC, Price JC, Greer P, McConaha C, Skovira K. Reduced 5-HT2A receptor binding after recovery from anorexia nervosa. Biol Psychiatry 2002;52:896-906.*

[10] *Sommer C. Serotonin in pain and analgesia: actions in the periphery. Mol Neurobiol 2004;30:117-125.*

[11] *Schwartz PJ, Wehr TA, Rosenthal NE, Bartko JJ, Oren DA, Luetke C, Murphy DL. Serotonin and thermoregulation. Physiologic and pharmacologic aspects of control revealed by intravenous m-CPP in normal human subjects. Neuropsychopharmacology 1995;13:105-115.*

[12] *Williams GV, Rao SG, Goldman-Rakic PS. The physiological role of 5-HT2A receptors in working memory. J Neurosci 2002;22:2843-2854.*

[13] *Endo T, Minami M, Hirafuji M, Ogawa T, Akita K, Nemoto M, Saito H, Yoshioka M, Parvez SH. Neurochemistry and neuropharmacology of emesis - the role of serotonin. Toxicology 2000;153:189-201.*

[14] *Frohlich PF, Meston CM. Evidence that serotonin affects female sexual functioning via peripheral mechanisms. Physiol Behav 2000;71:383-393.*

[15] *Blier P, de Montigny C, Chaput Y. Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. J Clin Psychopharmacol 1987;7:24S-35S.*

[16] *Blier P, Abbott FV. Putative mechanisms of action of antidepressant drugs in affective and anxiety disorders and pain. J Psychiatry Neurosci 2001;26:37-43.*

[17] *Delgado PL, Moreno FA. Hallucinogens, serotonin and obsessive-compulsive disorder. J Psychoactive Drugs 1998;30. 30:359-366, 359-366.*

[18] *Haugbol S, Pinborg LH, Regeur L, Hansen ES, Bolwig TG, Nielsen FA, Svarer C, Skovgaard LT, Knudsen GM. Cerebral 5-HT2A receptor binding is increased in patients with Tourette's syndrome. Int J Neuropsychopharmacol 2006:1-8.*

[19] *Meltzer HY. Role of serotonin in the action of atypical antipsychotic drugs. Clin Neurosci 1995;3:64-75.*

[20] *Nichols DE. Hallucinogens. Pharmacol Ther 2004;101:131-181.*

[21] *Hamon M, Bourgoin S. [Role of serotonin and other neuroactive molecules in the physiopathogenesis of migraine. Current hypotheses]. Pathol Biol (Paris) 2000;48:619-629.*

[22] *Newhouse P, Tatro A, Naylor M, Quealey K, Delgado P. Alzheimer disease, serotonin systems, and tryptophan depletion. Am J Geriatr Psychiatry 2002;10:483-484.*

[23] *Gerra G, Garofano L, Castaldini L, Rovetto F, Zaimovic A, Moi G, Bussandri M, Branchi B, Brambilla F, Friso G, Donnini C. Serotonin transporter promoter polymorphism genotype is associated with temperament, personality traits and illegal drugs use among adolescents. J Neural Transm 2005;112:1397-1410.*

[24] *Moresco FM, Dieci M, Vita A, Messa C, Gobbo C, Galli L, Rizzo G, Panzacchi A, De Peri L, Invernizzi G, Fazio F. In vivo serotonin 5HT(2A) receptor binding and personality traits in healthy subjects: a positron emission tomography study. Neuroimage 2002;17:1470-1478.*

[25] *Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386-389.*

[26] *Wagner KD, Robb AS, Findling RL, Jin J, Gutierrez MM, Heydorn WE. A randomized, placebo-controlled trial of citalopram for the treatment of major depression in children and adolescents. Am J Psychiatry 2004;161:1079-1083.*

[27] *Brody AL, Saxena S, Fairbanks LA, Alborzian S, Demaree HA, Maidment KM, Baxter LRJ. Personality changes in adult subjects with major depressive disorder or obsessive-compulsive disorder treated with paroxetine. J Clin Psychiatry 2000;61:349-355.*

[28] *Schooler N, Rabinowitz J, Davidson M, Emsley R, Harvey PD, Kopala L, McGorry PD, Van Hove I, Eerdekens M, Swyzen W, De Smedt G. Risperidone and haloperidol in first-episode psychosis: a long-term randomized trial. Am J Psychiatry 2005;162:947-953.*

[29] *Scahill L, Leckman JF, Schultz RT, Katsovich L, Peterson BS. A placebo-controlled trial of risperidone in Tourette syndrome. Neurology 2003;60:1130-1135.*

[30] *Damen L, Bruijn JK, Verhagen AP, Berger MY, Passchier J, Koes BW. Symptomatic treatment of migraine in children: a systematic review of medication trials. Pediatrics 2005;116:e295-302.*

[31] *Stenfors C, Ross SB. Changes in extracellular 5-HIAA concentrations as measured by in vivo microdialysis technique in relation to changes in 5-HT release. Psychopharmacology 2004;172. 172:119-128, 119-128. Epub 2004 Jan 2020.*

[32] *Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999;38. 38:1083-1152, 1083-1152.*

[33] *Jacobs BL, Azmitia EC. Structure and function of the brain serotonin system. Physiol 1992;72. 72:165-229, 165-229.*

[34] *Pineyro G, Blier P. Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacol Rev 1999;51:533-591.*

[35] *Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527-1531.*

[36] *Ito H, Nyberg S, Halldin C, Lundkvist C, Farde L. PET imaging of central 5-HT2A receptors with carbon-11-MDL 100,907. J Nucl Med 1998;39:208-214.*

[37] *Biver F, Goldman S, Luxen A, Monclus M, Forestini M, Mendlewicz J, Lotstra F. Multicompartmental study of fluorine-18 altanserin binding to brain 5HT2 receptors in humans using positron emission tomography. Eur 1994;21. 21:937-946, 937-946.*

[38] *Kristiansen H, Elfving B, Plenge P, Pinborg LH, Gillings N, Knudsen GM. Binding characteristics of the 5-HT2A receptor antagonists altanserin and MDL 100907. Synapse 2005;58:249-257.*

[39] *Price JC, Lopresti BJ, Meltzer CC, Smith GS, Mason NS, Huang Y, Holt DP, Gunn RN, Mathis CA. Analyses of [(18)F]altanserin bolus injection PET data. II: consideration of radiolabeled metabolites in humans. Synapse 2001;41. 41:11-21, 11-21.*

[40] *Pinborg LH, Adams KH, Svarer C, Holm S, Hasselbalch SG, Haugbol S, Madsen J, Knudsen GM. Quantification of 5-HT2A receptors in the human brain using [18F]altanserin-PET and the bolus/infusion approach. J Cereb Blood Flow Metab 2003;23:985-996.*

[41] *Smith GS, Price JC, Lopresti BJ, Huang Y, Simpson N, Holt D, Mason NS, Meltzer CC, Sweet RA, Nichols T, Sashin D, Mathis CA. Test-retest variability of serotonin 5-HT2A receptor binding measured with positron emission tomography and [18F]altanserin in the human brain. Synapse 1998;30. 30:380-392, 380-392.*

[42] *Azmitia EC, Segal M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 1978;179:641-667.*

[43] *Lopez-Gimenez JF, Mengod G, Palacios JM, Vilaro MT. Human striosomes are enriched in 5-HT2A receptors: autoradiographical visualization with [3H]MDL100,907,[125I](+/-)DOI and [3H]ketanserin. Eur J Neurosci 1999;11:3761-3765.*

[44] *Graybiel AM. Neural networks: neural systems V: basal ganglia. Am J Psychiatry 2001;158:21.*

[45] *Graybiel AM, Canales JJ. The neurobiology of repetitive behaviors: clues to the neurobiology of Tourette syndrome. Adv Neurol 2001;85:123-131.*

[46] *Willins DL, Deutch AY, Roth BL. Serotonin 5-HT2A receptors are expressed on pyramidal cells and interneurons in the rat cortex. Synapse 1997;27. 27:79-82, 79-82.*

[47] *Jakab RL, Goldman-Rakic PS. Segregation of serotonin 5-HT2A and 5-HT3 receptors in inhibitory circuits of the primate cerebral cortex. J Comp Neurol 2000;417:337-348.*

[48] *Aghajanian GK, Marek GJ. Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. Brain Res 1999;825:161-171.*

[49] *Ichikawa J, Meltzer HY. DOI, a 5-HT2A/2C receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. Brain Res 1995;698:204-208.*

[50] *Ikemoto K, Nishimura A, Okado N, Mikuni M, Nishi K, Nagatsu I. Human midbrain dopamine neurons express serotonin 2A receptor: an immunohistochemical demonstration. Brain Res 2000;853:377-380.*

[51] *Kroeze WK, Roth BL. The molecular biology of serotonin receptors: therapeutic implications for the interface of mood and psychosis. Biol Psychiatry 1998;44:1128-1142.*

[52] *Meltzer HY. The role of serotonin in antipsychotic drug action. Neuropsychopharmacology 1999;21:106S-115S.*

[53] *Ostroff RB, Nelson JC. Risperidone augmentation of selective serotonin reuptake inhibitors in major depression. J Clin Psychiatry 1999;60:256-259.*

[54] *Shelton RC, Tollefson GD, Tohen M, Stahl S, Gannon KS, Jacobs TG, Buras WR, Bymaster FP, Zhang W, Spencer KA, Feldman PD, Meltzer HY. A novel augmentation strategy for treating resistant major depression. Am J Psychiatry 2001;158:131-134.*

[55] *Marek GJ, Carpenter LL, McDougle CJ, Price LH. Synergistic action of 5-HT2A antagonists and selective serotonin reuptake inhibitors in neuropsychiatric disorders. Neuropsychopharmacology 2003;28:402-412.*

[56] *Gray JA, Roth BL. Paradoxical trafficking and regulation of 5-HT(2A) receptors by agonists and antagonists. Brain 2001;56. 56:441-451, 441-451.*

[57] *Arango V, Underwood MD, Mann JJ. Postmortem findings in suicide victims. Implications for in vivo imaging studies. Ann N Y Acad Sci 1997;836:269-287.*

[58] *Meyer JH, Kapur S, Houle S, DaSilva J, Owczarek B, Brown GM, Wilson AA, Kennedy SH. Prefrontal cortex 5-HT2 receptors in depression: an [18F]setoperone PET imaging study. Am J Psychiatry 1999;156:1029-1034.*

[59] *Attar-Levy D, Martinot JL, Blin J, Dao-Castellana MH, Crouzel C, Mazoyer B, Poirier MF, Bourdel MC, Aymard N, Syrota A, Feline A. The cortical serotonin2 receptors studied with positron-emission tomography and [18F]-setoperone during depressive illness and antidepressant treatment with clomipramine. Biol Psychiatry 1999;45:180-186.*

[60] *Meltzer CC, Price JC, Mathis CA, Greer PJ, Cantwell MN, Houck PR, Mulsant BH, Ben-Eliezer D, Lopresti B, DeKosky ST, Reynolds CF, 3rd. PET imaging of serotonin type 2A receptors in late-life neuropsychiatric disorders. Am J Psychiatry 1999;156:1871-1878.*

[61] *Meltzer HY, Arvanitis L, Bauer D, Rein W. Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. Am J Psychiatry 2004;161:975-984.*

[62] *Blier P, Szabo ST. Potential mechanisms of action of atypical antipsychotic medications in treatment-resistant depression and anxiety. J Clin Psychiatry 2005;66 Suppl 8:30-40.*

[63] *Adams KH, Hansen ES, Pinborg LH, Hasselbalch SG, Svarer C, Holm S, Bolwig TG, Knudsen GM. Patients with obsessive-compulsive disorder have increased 5-HT2A receptor binding in the caudate nuclei. Int J Neuropsychopharmacol 2005;8:391-401.*

[64] *Roth BL, Berry SA, Kroeze WK, Willins DL, Kristiansen K. Serotonin 5-HT2A receptors: molecular biology and mechanisms of regulation. Crit Rev Neurobiol 1998;12:319-338.*

[65] *Kroeze WK, Sheffler DJ, Roth BL. G-protein-coupled receptors at a glance. J Cell Sci 2003;116:4867-4869.*

[66] *Kenakin T. Efficacy at G-protein-coupled receptors. Nat Rev Drug Discov 2002;1:103-110.*

[67] *Egan C, Grinde E, Dupre A, Roth BL, Hake M, Teitler M, Herrick-Davis K. Agonist high and low affinity state ratios predict drug intrinsic activity and a revised ternary complex mechanism at serotonin 5-HT(2A) and 5-HT(2C) receptors. Synapse 2000;35. 35:144-150, 144-150.*

[68] *Van Oekelen D, Luyten WH, Leysen JE. 5-HT2A and 5-HT2C receptors and their atypical regulation properties. Life Sci 2003;72:2429-2449.*

[69] *Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev 2001;53:1-24.*

[70] *Gray JA, Bhatnagar A, Gurevich VV, Roth BL. The interaction of a constitutively active arrestin with the arrestin-insensitive 5-HT(2A) receptor induces agonist-independent internalization. Mol Pharmacol 2003;63. 63:961-972, 961-972.*

[71] *Zhou FC, Sari Y, Zhang JK. Expression of serotonin transporter protein in developing rat brain. Brain Res Dev Brain Res 2000;119:33-45.*

[72] *Sundstrom E, Kolare S, Souverbie F, Samuelsson EB, Pschera H, Lunell NO, Seiger A. Neurochemical differentiation of human bulbospinal monoaminergic neurons during the first trimester. Brain Res Dev Brain Res 1993;75:1-12.*

[73] *Lauder JM. Ontogeny of the serotonergic system in the rat: serotonin as a developmental signal. Ann N Y Acad Sci 1990;600:297-313; discussion 314.*

[74] *Lesch KP, Mossner R. Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? Biol Psychiatry 1998;44:179-192.*

[75] *Brezun JM, Daszuta A. Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafted with foetal raphe neurons. Eur J Neurosci 2000;12:391-396.*

[76] *Brezun JM, Daszuta A. Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. Neuroscience 1999;89:999-1002.*

[77] *Jennings KA, Loder MK, Sheward WJ, Pei Q, Deacon RM, Benson MA, Olverman HJ, Hastie ND, Harmar AJ, Shen S, Sharp T. Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. J Neurosci 2006;26:8955-8964.*

[78] *Turecki G, Briere R, Dewar K, Antonetti T, Lesage AD, Seguin M, Chawky N, Vanier C, Alda M, Joober R, Benkelfat C, Rouleau GA. Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide. Am J Psychiatry 1999;156:1456-1458.*

[79] *Hernandez I, Sokolov BP. Abnormalities in 5-HT2A receptor mRNA expression in frontal cortex of chronic elderly schizophrenics with varying histories of neuroleptic treatment. J Neurosci Res 2000;59:218-225.*

[80] *Williams J, Spurlock G, McGuffin P, Mallet J, Nothen MM, Gill M, Aschauer H, Nylander PO, Macciardi F, Owen MJ. Association between schizophrenia and T102C polymorphism of the 5-hydroxytryptamine type 2a-receptor gene. European Multicentre Association Study of Schizophrenia (EMASS) Group. Lancet 1996;347:1294-1296.*

[81] *Arranz MJ, Munro J, Owen MJ, Spurlock G, Sham PC, Zhao J, Kirov G, Collier DA, Kerwin RW. Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT2A receptor gene and response to clozapine. Mol Psychiatry 1998;3:61-66.*

[82] *Bradley SL, Dodelzon K, Sandhu HK, Philibert RA. Relationship of serotonin transporter gene polymorphisms and haplotypes to mRNA transcription. Am J Med Genet B Neuropsychiatr Genet 2005;136:58-61.*

[83] *Yu YW, Tsai SJ, Chen TJ, Lin CH, Hong CJ. Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders. Mol Psychiatry 2002;7:1115-1119.*

[84] *Malhotra AK, Murphy GM, Jr., Kennedy JL. Pharmacogenetics of psychotropic drug response. Am J Psychiatry 2004;161:780-796.*

[85] *Fiskerstrand CE, Lovejoy EA, Quinn JP. An intronic polymorphic domain often associated with susceptibility to affective disorders has allele dependent differential enhancer activity in embryonic stem cells. FEBS Lett 1999;458:171-174.*

[86] *MacKenzie A, Quinn J. A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. Proc Natl Acad Sci U S A 1999;96:15251-15255.*

[87] *Mellerup E, Bennike B, Bolwig T, Dam H, Hasholt L, Jorgensen MB, Plenge P, Sorensen SA. Platelet serotonin transporters and the transporter gene in control subjects, unipolar patients and bipolar patients. Acta Psychiatr Scand 2001;103:229-233.*

[88] *Gutierrez B, Pintor L, Gasto C, Rosa A, Bertranpetit J, Vieta E, Fananas L. Variability in the serotonin transporter gene and increased risk for major depression with melancholia. Hum Genet 1998;103:319-322.*

[89] *Fan JB, Sklar P. Meta-analysis reveals association between serotonin transporter gene STin2 VNTR polymorphism and schizophrenia. Mol Psychiatry 2005;10:928-938, 891.*

[90] *Lotrich FE, Pollock BG. Meta-analysis of serotonin transporter polymorphisms and affective disorders. Psychiatr Genet 2004;14:121-129.*

[91] *Leckman JF. Tourette's syndrome. Lancet 2002;360:1577-1586.*

[92] *Leckman JF, Bloch MH, Scahill L, King RA. Tourette syndrome: the self under siege. J Child Neurol 2006;21:642-649.*

[93] *Leckman JF, Walker DE, Goodman WK, Pauls DL, Cohen DJ. "Just right" perceptions associated with compulsive behavior in Tourette's syndrome. Am 1994;151. 151:675-680, 675-680.*

[94] *Robertson MM. Tourette syndrome, associated conditions and the complexities of treatment. Brain 2000;123 Pt 3:425-462.*
[95] *Kerbeshian J, Burd L. Epidemiology and comorbidity. The North Dakota prevalence studies of Tourette syndrome and other developmental disorders. Adv Neurol 1992;58. 58:67-74.:67-74.*
[96] *Jankovic J. Tourette syndrome. Phenomenology and classification of tics. Neurol Clin 1997;15. 15:267-275, 267-275.*
[97] *Abuzzahab FE, Sr., Anderson FO. Gilles de la Tourette's syndrome; international registry. Minn Med 1973;56:492-496.*
[98] *Caine ED, McBride MC, Chiverton P, Bamford KA, Rediess S, Shiao J. Tourette's syndrome in Monroe County school children. Neurology 1988;38:472-475.*
[99] *Comings DE, Himes JA, Comings BG. An epidemiologic study of Tourette's syndrome in a single school district. J Clin Psychiatry 1990;51:463-469.*
[100] *Apter A, Pauls DL, Bleich A, Zohar AH, Kron S, Ratzoni G, Dycian A, Kotler M, Weizman A, Gadot N, et al. An epidemiologic study of Gilles de la Tourette's syndrome in Israel. Arch Gen Psychiatry 1993;50:734-738.*
[101] *Robertson MM, Boardman J. Tourette's syndrome in the year 2000. Aust N Z J Psychiatry 1996;30:749-759.*
[102] *Costello EJ, Angold A, Burns BJ, Stangl DK, Tweed DL, Erkanli A, Worthman CM. The Great Smoky Mountains Study of Youth. Goals, design, methods, and the prevalence of DSM-III-R disorders. Arch Gen Psychiatry 1996;53:1129-1136.*
[103] *Kadesjo B, Gillberg C. Tourette's disorder: epidemiology and comorbidity in primary school children. J Am Acad Child Adolesc Psychiatry 2000;39:548-555.*
[104] *Hornsey H, Banerjee S, Zeitlin H, Robertson MM. The Prevalence of Tourette's Syndrome in 13-14-olds in Mainstream Schools. Journal of Child Psychology and Psychiatry 2001;42:1035-1039.*
[105] *Price RA, Kidd KK, Cohen DJ, Pauls DL, Leckman JF. A twin study of Tourette syndrome. Arch Gen Psychiatry 1985;42:815-820.*
[106] *Abelson JF, Kwan KY, O'Roak BJ, Baek DY, Stillman AA, Morgan TM, Mathews CA, Pauls DL, Rasin MR, Gunel M, Davis NR, Ercan-Sencicek AG, Guez DH, Spertus JA, Leckman JF, Dure LSt, Kurlan R, Singer HS, Gilbert DL, Farhi A, Louvi A, Lifton RP, Sestan N, State MW. Sequence variants in SLITRK1 are associated with Tourette's syndrome. Science 2005;310:317-320.*
[107] *Frey KA, Albin RL. Neuroimaging of Tourette syndrome. J Child Neurol 2006;21:672-677.*
[108] *Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 1986;9:357-381.*
[109] *Leckman JF, Peterson BS, Anderson GM, Arnsten AF, Pauls DL, Cohen DJ. Pathogenesis of Tourette's syndrome. J Child Psychol Psychiatry 1997;38:119-142.*
[110] *Peterson BS, Skudlarski P, Anderson AW, Zhang H, Gatenby JC, Lacadie CM, Leckman JF, Gore JC. A functional magnetic resonance imaging study of tic suppression in Tourette syndrome. Arch Gen Psychiatry 1998;55:326-333.*
[111] *Mink JW. Neurobiology of basal ganglia and Tourette syndrome: basal ganglia circuits and thalamocortical outputs. Adv Neurol 2006;99:89-98.*
[112] *Shapiro E, Shapiro AK, Fulop G, Hubbard M, Mandeli J, Nordlie J, Phillips RA. Controlled study of haloperidol, pimozide and placebo for the treatment of Gilles de la Tourette's syndrome. Arch Gen Psychiatry 1989;46:722-730.*
[113] *Regeur L, Pakkenberg B, Fog R, Pakkenberg H. Clinical features and long-term treatment with pimozide in 65 patients with Gilles de la Tourette's syndrome. J Neurol Neurosurg Psychiatry 1986;49:791-795.*
[114] *Jacobs BL, Trulson ME, Heym J, Steinfels GF. On the role of CNS serotonin in the motor abnormalities of Tourette syndrome: behavioral and single-unit studies. Adv Neurol 1982;35. 35:93-8.:93-98.*
[115] *Jacobs BL, Fornal CA. 5-HT and motor control: a hypothesis. Trends Neurosci 1993;16. 16:346-352, 346-352.*
[116] *Blier P, Habib R, Flament MF. Pharmacotherapies in the management of obsessive-compulsive disorder. Can J Psychiatry 2006;51:417-430.*
[117] *Scahill L, Riddle MA, King RA, Hardin MT, Rasmusson A, Makuch RW, Leckman JF. Fluoxetine has no marked effect on tic symptoms in patients with Tourette's syndrome: a double-blind placebo-controlled study. J Child Adolesc Psychopharmacol 1997;7:75-85.*
[118] *Comings DE. Blood serotonin and tryptophan in Tourette syndrome. Am J Med Genet 1990;36:418-430.*

[119] Anderson GM, Pollak ES, Chatterjee D, Leckman JF, Riddle MA, Cohen DJ. Postmortem analysis of subcortical monoamines and amino acids in Tourette syndrome. Adv Neurol 1992;58:123-133.

[120] Butler IJ, Koslow SH, Seifert WEJ, Caprioli RM, Singer HS. Biogenic amine metabolism in Tourette syndrome. Ann Neurol 1979;6. 6:37-39, 37-39.

[121] Leckman JF, Anderson GM, Cohen DJ, Ort S, Harcherik DF, Hoder EL, Shaywitz BA. Whole blood serotonin and tryptophan levels in Tourette's disorder: effects of acute and chronic clonidine treatment. Life Sci 1984;35:2497-2503.

[122] Leckman JF, Goodman WK, Anderson GM, Riddle MA, Chappell PB, McSwiggan-Hardin MT, McDougle CJ, Scahill LD, Ort SI, Pauls DL, et al. Cerebrospinal fluid biogenic amines in obsessive compulsive disorder, Tourette's syndrome, and healthy controls. Neuropsychopharmacology 1995;12:73-86.

[123] Heinz A, Knable MB, Wolf SS, Jones DW, Gorey JG, Hyde TM, Weinberger DR. Tourette's syndrome: [I-123]beta-CIT SPECT correlates of vocal tic severity. Neurology 1998;51:1069-1074.

[124] Muller-Vahl KR, Meyer GJ, Knapp WH, Emrich HM, Gielow P, Brucke T, Berding G. Serotonin transporter binding in Tourette Syndrome. Neurosci Lett 2005;385:120-125.

[125] Bishop C, Tessmer JL, Ullrich T, Rice KC, Walker PD. Serotonin 5-HT2A receptors underlie increased motor behaviors induced in dopamine -depleted rats by intrastriatal 5-HT2A/2C agonism. J Pharmacol Exp Ther 2004;310:687-694.

[126] Kuroki T, Meltzer HY, Ichikawa J. 5-HT 2A receptor stimulation by DOI, a 5-HT 2A/2C receptor agonist, potentiates amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. Brain Res 2003;972. 972:216-221, 216-221.

[127] Higgins GA, Enderlin M, Haman M, Fletcher PJ. The 5-HT2A receptor antagonist M100,907 attenuates motor and 'impulsive-type' behaviours produced by NMDA receptor antagonism. Psychopharmacology (Berl) 2003;170:309-319.

[128] Ninan I, Kulkarni SK. 5-HT2A receptor antagonists block MK-801-induced stereotypy and hyperlocomotion. Eur J Pharmacol 1998;358:111-116.

[129] Sallee FR, Kurlan R, Goetz CG, Singer H, Scahill L, Law G, Dittman VM, Chappell PB. Ziprasidone treatment of children and adolescents with Tourette's syndrome: a pilot study. J Am Acad Child Adolesc Psychiatry 2000;39:292-299.

[130] Onofrj M, Paci C, D'Andreamatteo G, Toma L. Olanzapine in severe Gilles de la Tourette syndrome: a 52-week double-blind cross-over study vs. low-dose pimozide. J 2000;247. 247:443-446, 443-446.

[131] Bonnier C, Nassogne MC, Evrard P. Ketanserin treatment of Tourette's syndrome in children. Am J Psychiatry 1999;156:1122-1123.

[132] Leysen JE. 5-HT(2) Receptors. Curr Drug Target CNS Neurol Disord 2004;3. 3:11-26, 11-26.

[133] Smeraldi E, Zanardi R, Benedetti F, Di Bella D, Perez J, Catalano M. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. Mol Psychiatry 1998;3:508-511.

[134] Muller-Gartner HW, Links JM, Prince JL, Bryan RN, McVeigh E, Leal JP, Davatzikos C, Frost JJ. Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. J Cereb Blood Flow Metab 1992;12:571-583.

[135] Tan PZ, Baldwin RM, Van Dyck CH, Al-Tikriti M, Roth B, Khan N, Charney DS, Innis RB. Characterization of radioactive metabolites of 5-HT2A receptor PET ligand [18F]altanserin in human and rodent. Nucl Med Biol 1999;26:601-608.

[136] Forutan F, Estalji S, Beu M, Nikolaus S, Hamacher K, Coenen HH, Vosberg H, Muller-Gartner HW, Larisch R. Distribution of 5HT2A receptors in the human brain: comparison of data in vivo and post mortem. Nuklearmedizin 2002;41:197-201.

[137] Willendrup P, Pinborg LH, Hasselbalch SG, Adams KH, Stahr K, Knudsen GM, Svarer C. Assessment of the precision in co-registration of structural MR-images and PET-images with localized binding. In: Iida H. Shah NJ. Hayashi T. and Watabe H. Quantification in Biomedical Imaging with PET and MRI. International Congress Series. 2004;1265:275-280.

[138] Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, Holm S, Paulson OB, Knudsen GM. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. Neuroimage 2005;24:969-979.

[139] Mueller-Gaertner HW, Links JM, Prince JL, Bryan RN, McVeigh E, Leal JP, Davatzikos C, Frost JJ. Measurement of radiotracer concentration in brain gray matter using positron

emission tomography: MRI-based correction for partial volume effects. J Cereb Blood Flow Metab 1992;12:571-583.

[140] Quarantelli M, Berkouk K, Prinster A, Landeau B, Svarer C, Balkay L, Alfano B, Brunetti A, Baron JC, Salvatore M. Integrated software for the analysis of brain PET/SPECT studies with partial-volume-effect correction. J Nucl Med 2004;45:192-201.

[141] Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S, Madsen K, Frokjaer V, Martiny L, Paulson OB, Knudsen GM. A database of [(18)F]-altanserin binding to 5-HT(2A) receptors in normal volunteers: normative data and relationship to physiological and demographic variables. Neuroimage 2004;21:1105-1113.

[142] Frokjaer VG, Mortensen EL, Adams KH, Haugbøl S, Pinborg LH, Nielsen FA, Svarer C, Hasselbalch SG, Holm S, Paulson OB, Knudsen GM. Frontolimbic serotonin 2A receptor binding in healthy subjects is associated with personality traits predisposing to affective disorder. Submitted 2006.

[143] Hansen HS, Mortensen EL, Schiøtz HK. Dokumentation for den danske udgave af NEO PI-R og NEO PI-R kort Version. . Dansk psykologisk forlag,Copenhagen, Denmark,2004.

[144] Ohara K, Nagai M, Tsukamoto T, Tani K, Suzuki Y, Ohara K. 5-HT2A receptor gene promoter polymorphism--1438G/A and mood disorders. Neuroreport 1998;9:1139-1141.

[145] Arranz M, Collier D, Sodhi M, Ball D, Roberts G, Price J, Sham P, Kerwin R. Association between clozapine response and allelic variation in 5-HT2A receptor gene. Lancet 1995;346:281-282.

[146] Arranz MJ, Collier DA, Munro J, Sham P, Kirov G, Sodhi M, Roberts G, Price J, Kerwin RW. Analysis of a structural polymorphism in the 5-HT2A receptor and clinical response to clozapine. Neurosci Lett 1996;217:177-178.

[147] Ogilvie AD, Battersby S, Bubb VJ, Fink G, Harmar AJ, Goodwim GM, Smith CA. Polymorphism in serotonin transporter gene associated with susceptibility to major depression. Lancet 1996;347:731-733.

[148] Masellis M, Basile V, Meltzer HY, Lieberman JA, Sevy S, Macciardi FM, Cola P, Howard A, Badri F, Nothen MM, Kalow W, Kennedy JL. Serotonin subtype 2 receptor genes and clinical response to clozapine in schizophrenia patients. Neuropsychopharmacology 1998;19:123-132.

[149] Quist JF, Barr CL, Schachar R, Roberts W, Malone M, Tannock R, Basile VS, Beitchman J, Kennedy JL. Evidence for the serotonin HTR2A receptor gene as a susceptibility factor in attention deficit hyperactivity disorder (ADHD). Mol Psychiatry 2000;5:537-541.

[150] Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621-2624.

[151] Hranilovic D, Stefulj J, Schwab S, Borrmann-Hassenbach M, Albus M, Jernej B, Wildenauer D. Serotonin transporter promoter and intron 2 polymorphisms: relationship between allelic variants and gene expression. Biol Psychiatry 2004;55:1090-1094.

[152] Edgington ES. Randomization tests. New York: Marcel Dekker,1995.

[153] Lovejoy EA, Scott AC, Fiskerstrand CE, Bubb VJ, Quinn JP. The serotonin transporter intronic VNTR enhancer correlated with a predisposition to affective disorders has distinct regulatory elements within the domain based on the primary DNA sequence of the repeat unit. Eur J Neurosci 2003;17:417-420.

[154] Soeby K, Larsen SA, Olsen L, Rasmussen HB, Werge T. Serotonin transporter: evolution and impact of polymorphic transcriptional regulation. Am J Med Genet B Neuropsychiatr Genet 2005;136:53-57.

[155] Buckholtz NS, Zhou DF, Freedman DX. Serotonin2 agonist administration down-regulates rat brain serotonin2 receptors. Life Sci 1988;42:2439-2445.

[156] Heinz A, Jones DW, Mazzanti C, Goldman D, Ragan P, Hommer D, Linnoila M, Weinberger DR. A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. Biol Psychiatry 2000;47:643-649.

[157] David SP, Murthy NV, Rabiner EA, Munafo MR, Johnstone EC, Jacob R, Walton RT, Grasby PM. A functional genetic variation of the serotonin (5-HT) transporter affects 5-HT1A receptor binding in humans. J Neurosci 2005;25:2586-2590.

[158] Schinka JA, Busch RM, Robichaux-Keene N. A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. Mol Psychiatry 2004;9:197-202.

[159] Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT. Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. Am J Med Genet B Neuropsychiatr Genet 2005;133:110-115.

[160] Gillespie NA, Whitfield JB, Williams B, Heath AC, Martin NG. The relationship between stressful life events, the serotonin transporter (5-HTTLPR) genotype and major depression. Psychol Med 2005;35:101-111.

[161] Biver F, Wikler D, Lotstra F, Damhaut P, Goldman S, Mendlewicz J. Serotonin 5-HT2 receptor imaging in major depression: focal changes in orbito-insular cortex. Br J Psychiatry 1997;171:444-448.

[162] Yatham LN, Liddle PF, Shiah IS, Scarrow G, Lam RW, Adam MJ, Zis AP, Ruth TJ. Brain serotonin2 receptors in major depression: a positron emission tomography study. Arch Gen Psychiatry 2000;57:850-858.

[163] Ngan ET, Yatham LN, Ruth TJ, Liddle PF. Decreased serotonin 2A receptor densities in neuroleptic-naive patients with schizophrenia: A PET study using [(18)F]setoperone. Am J Psychiatry 2000;157:1016-1018.

[164] Meyer JH, Kapur S, Eisfeld B, Brown GM, Houle S, DaSilva J, Wilson AA, Rafi-Tari S, Mayberg HS, Kennedy SH. The effect of paroxetine on 5-HT(2A) receptors in depression: an [(18)F]setoperone PET imaging study. Am J Psychiatry 2001;158. 158:78-85, 78-85.

[165] Dhaenen H. Imaging the serotonergic system in depression. Eur Arch Psychiatry Clin Neurosci 2001;251 Suppl 2:II76-80.

[166] Trichard C, Paillere-Martinot ML, Attar-Levy D, Blin J, Feline A, Martinot JL. No serotonin 5-HT2A receptor density abnormality in the cortex of schizophrenic patients studied with PET. Schizophr Res 1998;31:13-17.

[167] Verhoeff NP, Meyer JH, Kecojevic A, Hussey D, Lewis R, Tauscher J, Zipursky RB, Kapur S. A voxel-by-voxel analysis of [18F]setoperone PET data shows no substantial serotonin 5-HT(2A) receptor changes in schizophrenia. Psychiatry Res 2000;99. 99:123-135, 123-135.

[168] Pinborg LH, Arfan HM, Haugbøl S, Kyvik KO, Hjelmborg JVB, Svarer C, Frokjaer VG, Paulson OB, Holm S, Knudsen GM. The density of 5-HT2A receptors in the human brain is strongly genetically determined. Submitted.

[169] Bray NJ, Buckland PR, Hall H, Owen MJ, O'Donovan MC. The serotonin-2A receptor gene locus does not contain common polymorphism affecting mRNA levels in adult brain. Mol Psychiatry 2004;9:109-114.

[170] Polesskaya OO, Sokolov BP. Differential expression of the "C" and "T" alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. J Neurosci Res 2002;67:812-822.

[171] Pompeiano M, Palacios JM, Mengod G. Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. Brain Res Mol Brain Res 1994;23:163-178.

[172] Polesskaya OO, Aston C, Sokolov BP. Allele C-specific methylation of the 5-HT2A receptor gene: evidence for correlation with its expression and expression of DNA methylase DNMT1. J Neurosci Res 2006;83:362-373.

[173] Kouzmenko AP, Hayes WL, Pereira AM, Dean B, Burnet PW, Harrison PJ. 5-HT2A receptor polymorphism and steady state receptor expression in schizophrenia. Lancet 1997;349:1815.

[174] Kouzmenko AP, Scaffidi A, Pereira AM, Hayes WL, Copolov DL, Dean B. No correlation between A(-1438)G polymorphism in 5-HT2A receptor gene promoter and the density of frontal cortical 5-HT2A receptors in schizophrenia. Hum Hered 1999;49:103-105.

[175] Haugbøl S, Pinborg LH, Haroon AM, Frøkjær V, Madsen J, Dyrby TB, Svarer C, Knudsen GM. Reproducibility and sample size estimation of 5-HT2A receptor measurements with [18F]altanserin PET bolus plus infusion approach. European Journal of Nuclear Medicine and Molecular Imaging 2006;(Accepted Sep 21 2006).

[176] Saka E, Graybiel AM. Pathophysiology of Tourette's syndrome: striatal pathways revisited. Brain Dev 2003;25 Suppl 1:S15-19.

[177] Smith GS, Dewey SL, Brodie JD, Logan J, Vitkun SA, Simkowitz P, Schloesser R, Alexoff DA, Hurley A, Cooper T, Volkow ND. Serotonergic modulation of dopamine measured with [11C]raclopride and PET in normal human subjects. Am J Psychiatry 1997;154:490-496.

[178] Vollenweider FX, Vontobel P, Hell D, Leenders KL. 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man--a PET study with [11C]raclopride. Neuropsychopharmacology 1999;20:424-433.

[179] Singer HS, Szymanski S, Giuliano J, Yokoi F, Dogan AS, Brasic JR, Zhou Y, Grace AA, Wong DF. Elevated intrasynaptic dopamine release in Tourette's syndrome measured by PET. Am J Psychiatry 2002;159:1329-1336.

[180] Pinborg LH, Adams KH, Yndgaard S, Hasselbalch SG, Holm S, Kristiansen H, Paulson OB, Knudsen GM. [18F]altanserin binding to human 5HT2A receptors is unaltered after citalopram and pindolol challenge. J Cereb Blood Flow Metab 2004;24:1037-1045.

[181] Eapen V, Trimble MR, Robertson MM. The use of fluoxetine in Gilles de la Tourette syndrome and obsessive compulsive behaviours: preliminary clinical experience. Prog Neuropsychopharmacol Biol Psychiatry 1996;20. 20:737-743, 737-743.

[182] Houle S, Ginovart N, Hussey D, Meyer JH, Wilson AA. Imaging the serotonin transporter with positron emission tomography: initial human studies with [11C]DAPP and [11C]DASB. Eur J Nucl Med 2000;27:1719-1722.

[183] Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, Suhara T, Suzuki K, Innis RB, Carson RE. Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. J Cereb Blood Flow Metab 2003;23:1096-1112.

Appendices

I. Reproducibility of 5-HT(2A) receptor measurements and sample size estimations with [(18)F]altanserin PET using a bolus/infusion approach.

II. A serotonin transporter polymorphism influence *in vivo* 5-HT_{2A} receptor binding in the brain

III. Cerebral 5-HT_{2A} receptor binding is increased in patients with Tourette's syndrome

Paper I

Eur J Nucl Med Mol Imaging
DOI 10.1007/s00259-006-0296-y

ORIGINAL ARTICLE

Reproducibility of 5-HT_{2A} receptor measurements and sample size estimations with [^{18}F]altanserin PET using a bolus/infusion approach

Steven Haugbøl · Lars H. Pinborg · Haroon M Arfan · Vibe M. Frøkjær · Jacob Madsen · Tim B. Dyrby · Claus Svarer · Gitte M. Knudsen

Received: 1 May 2006 / Accepted: 20 September 2006
© Springer-Verlag 2006

Abstract
Purpose To determine the reproducibility of measurements of brain 5-HT_{2A} receptors with an [^{18}F]altanserin PET bolus/infusion approach. Further, to estimate the sample size needed to detect regional differences between two groups and, finally, to evaluate how partial volume correction affects reproducibility and the required sample size.
Methods For assessment of the variability, six subjects were investigated with [^{18}F]altanserin PET twice, at an interval of less than 2 weeks. The sample size required to detect a 20% difference was estimated from [^{18}F]altanserin PET studies in 84 healthy subjects. Regions of interest were automatically delineated on co-registered MR and PET images.
Results In cortical brain regions with a high density of 5-HT_{2A} receptors, the outcome parameter (binding potential, BP_1) showed high reproducibility, with a median difference between the two group measurements of 6% (range 5–12%), whereas in regions with a low receptor density, BP_1 reproducibility was lower, with a median difference of 17% (range 11–39%). Partial volume correction reduced the variability in the sample considerably. The sample size required to detect a 20% difference in brain regions with high receptor density is approximately 27, whereas for low receptor binding regions the required sample size is substantially higher.
Conclusion This study demonstrates that [^{18}F]altanserin PET with a bolus/infusion design has very low variability, particularly in larger brain regions with high 5-HT_{2A} receptor density. Moreover, partial volume correction considerably reduces the sample size required to detect regional changes between groups.

Keywords Altanserin · Reliability · Sample size estimation · Serotonin receptor · Partial volume correction

S. Haugbøl (✉) · L. H. Pinborg · H. M. Arfan · V. M. Frøkjær · C. Svarer · G. M. Knudsen
Neurobiology Research Unit, Centre for Integrated Molecular Brain Imaging, Copenhagen University Hospital,
9 Blegdamsvej,
2100 Copenhagen, Denmark
e-mail: steven@nru.dk

J. Madsen
PET and Cyclotron Unit, Copenhagen University Hospital, Copenhagen, Denmark

T. B. Dyrby
Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital, Copenhagen, Denmark

Introduction

During recent decades, imaging of the serotonergic transmitter system in the living human brain has become possible by the use of radiolabelled tracers and positron emission tomography (PET). This has enabled studies to be conducted in order to elucidate the possible dysfunction of the serotonergic system in neuropsychiatric disorders. Whereas early studies of the 5-HT_{2A} receptors involved the use of less selective 5-HT_{2A} receptor tracers, such as [^{18}F]setoperone and [^{11}C]*N*-methyl-spiperone, the more recently developed 5-HT_{2A} receptor tracer [^{11}C]MDL100907 [1] and [^{18}F]altanserin [2] yield a specific signal with a high target-to-background ratio. These two tracers possess similar binding characteristics [3] but quantification of [^{18}F]altanserin binding is complicated by

the systemic production of radiolabelled metabolites that cross the blood-brain barrier. Several studies have tried to overcome the metabolite issue by sophisticated kinetic modelling where the kinetics of the radiolabelled compounds are taken into account [4]. Use of a bolus/infusion [^{18}F]altanserin protocol does, however, allow for reliable subtraction of the non-specific binding of radiotracer [5]. Reproducibility of altanserin PET measurements with bolus administration has previously been evaluated in a test–retest study [6], but there has been no thorough analysis of the individual sources of variation (plasma free fraction, non-specific binding, etc.). Moreover, the investigators used large hand-drawn regions, some of which covered both hemispheres and therefore were unsuitable for interpolation to clinical studies, given that such studies usually address more specific areas of the brain. In this study we evaluated the test–retest variability of [^{18}F]altanserin PET measurements with a bolus/infusion design in six healthy subjects in whom regions of interest (ROIs) were automatically delineated and subsequently partial volume corrected. In addition to these analyses, we investigated the reproducibility of the outcome measure, binding potential (BP_1), for brain 5-HT_{2A} receptors and accompanying values for non-specific binding, metabolite profile and plasma free radiotracer fraction (f_1). Lastly, a large sample of 84 subjects constituted the basis for conducting a power analysis to be used in the design of future [^{18}F]altanserin PET bolus/infusion experiments. We hypothesised that bolus/infusion administration together with automated ROI delineation yields a robust and reproducible estimate of 5-HT_{2A} receptor binding in the human brain. We further hypothesised that a correction for partial volume estimation (PVE), by taking into account the various degrees of brain atrophy across the entire age range, would reduce the interindividual variability and thereby reduce the required sample size in clinical PET investigations.

Materials and methods

To investigate the reproducibility of the method, we investigated six healthy Caucasian men (aged between 33 and 67 years) twice. Each person underwent MRI scanning once and PET scanning twice at an interval of 2–14 days. In addition, to generate means and standard deviations of regional binding potentials for power calculations and sample size estimations, data from a large group of healthy controls (n=84, 51 men, age range 18–74 years) were included. This sample also included the first set of PET images from the six subjects investigated in the test–retest study. Data on a part of this group (n=52) have previously been reported by Adams et al. [7]. None of the subjects had a history of neurological or psychiatric disease and they all had a normal neurological examination on the day of PET scanning. The protocol was approved by the Ethics Committee of Copenhagen and Frederiksberg [(KF) 02-058/99 and (KF) 12-142/03]. Written informed consent was obtained before inclusion.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) was performed on a 1.5-T Vision scanner (Siemens, Erlangen, Germany) where 158 slices were acquired continuously in the sagittal plane using a 3D MPRAGE sequence (TE 4 ms, TR 11 ms, TI 300 ms, flip angle 12°, image matrix 256×256, slice thickness 1.14 mm, in-plane resolution 1.21×1.21 mm).

[^{18}F]altanserin PET imaging

[^{18}F]altanserin PET scanning was conducted as described by Pinborg et al. [5]. [^{18}F]altanserin was administered as a combination of a bolus injection and a continuous infusion (ratio 1.75 h, i.e. the amount of tracergiven for 1.75 h by constant infusion was equal to that of the bolus) to obtain tracer steady state in blood and tissue. Subjects received a max. dose of 3.7 MBq/kg body weight [^{18}F]altanserin, with an average dose of 270 MBq. PET scans were performed with an 18-ring GE-Advance scanner (General Electric, Milwaukee, WI, USA) operating in 3D acquisition mode, producing 35 image slices with an interslice distance of 4.25 mm. The technical specifications have been described elsewhere [8, 9]. Dynamic 3D emission scans (five frames of 8 min each) started 120 min after administration of [^{18}F]altanserin. The five dynamic frames were aligned and resliced using the approach from Woods et al. [10], and subsequently an average image was generated. For all subjects, blood was sampled manually at mid PET frame times and analysed with high-performance liquid chromatography (HPLC) for determination of the activity of the non-metabolised tracer in plasma (free fraction, f_1). Visual inspection of the time-activity curves was done to assure constant blood and tissue levels.

Determination of parent compound in plasma

The plasma metabolites of [^{18}F]altanserin were determined using a modification of previously published procedures [11–13]. Plasma whole blood samples (13 ml) were centrifuged for 10 min at 4,700 g. Plasma (0.5 ml) was counted in a gamma counter (Cobra 5003, Packard Instruments, Meriden, CT, USA) for determination of radioactivity. Five millilitres of plasma was mixed with acetic acid (20 ml, 50 mmol/l) and eluted through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA, USA) which had been pre-activated with methanol (2 ml) and water

(10 ml). The Sep-Pak was washed with triethylamine (10 ml, 0.1%) and water (10 ml) before the outlet was connected to a filter (Millipore 0.45 μm), and the radioactive content was eluted with methanol (1 ml) followed by distilled water (3 ml). The resulting mixture (4 ml) was injected on the semipreparative HPLC system [column: Waters μBondapack C18, 7.8×300 mm; solvent A: CH_3COONH_4 (0.073 mol/l, adjusted to pH 4.5 with CH_3COOH); solvent B: 70% CH_3CN/30% CH_3COONH_4 (0.22 mol/l); flow: 6 ml/min] equipped with an ultraviolet detector (254 nm) and a Packard flow scintillation analyser (500TR series) connected in series. Solvent B was increased with a linear gradient from 25% to 100% for 10 min. The [^{18}F]altanserin eluted after 8.4 min and was well separated from the metabolites eluting after 5.1 min, 5.6 min and 7.3 min, respectively.

MRI/PET co-registration

MR images were co-registered using a Matlab-based (Mathworks Inc., Natick, MA, USA) interactive program which is based on visual identification of the transformation [14]. MR images were segmented into grey matter, white matter and cerebrospinal fluid tissue classes using overlay of prior probability images (SPM2). A total of 35 regions of interest (ROIs) were automatically delineated on MRI slices and transferred to PET images using the identified rigid body transformation [15]. PET images were PVE corrected using the segmented MRI. A three-compartment model based on grey matter, white matter and cerebrospinal fluid was used [16, 17]. The white matter value was extracted as the average voxel value from a white matter ROI (midbrain) in the uncorrected PET image.

The volume of distribution (V_d) and binding potential (BP_1 and BP) were calculated for each ROI on the basis of the individual mean count density within each ROI (C_{ROI}), the individual cerebellum ($C_{cerebellum}$), representing non-specific binding (NS) [5], and the metabolite-corrected plasma concentration of [^{18}F]altanserin (C_{plasma}): $V_{d(ROI)} = C_{ROI}/C_{plasma,}\ V_{d(NS)} = C_{cerebellum}/C_{plasma,}\ BP_1 = [V_{d(ROI)} - V_{d(NS)}]$ and $BP = [V_{d(ROI)} - V_{d(NS)}/f_1]$ where f_1 is the free fraction of parent compound in plasma. Data were analysed for a total of 17 cortical and subcortical ROIs in the left hemisphere representing both high and low tracer binding (Table 1).

Reproducibility and sample size estimation

To examine the reproducibility of the prime outcome parameter, BP_1, and its components (non-specific binding, plasma concentrations, parent compound, and also f_1), differences were calculated as: variability = absolute(test−*retest*)/((test + retest) × 0.5). To estimate the correlation between repeated measurements in the same subject, we calculated the intraclass correlation coefficient (ICC): $ICC = (MS_{between} - MS_{within})/(MS_{between} + (m-1) \times MS_{within})$ (statistical software package, SPSS) where m is the number of observations per subject, $MS_{between}$ is the mean sum of squares between subjects and MS_{within} is the mean sum of squares within subjects. The variance of components was estimated from one-way analysis of variance.

Sample size estimates were calculated from mean and SD of BP_1 estimated in each ROI and were based on the sample size required to detect a difference of 20% between two independent groups with a significance level (α) of 0.05 and a power (β) of 0.8 (Analyst; statistical software package, SAS). The age effect on [^{18}F]altanserin binding was taken into account by using the SD of the residuals from a simple regression model: residual=BP_1−(age×slope+ intercept), where slope and intercept were calculated from the sample of 84 subjects.

Results

Table 1 shows median percent differences and intraclass correlation resulting from the two examinations. As expected, there was no significant difference in the f_1 (the free fraction of parent compound in plasma) or in the non-specific binding between the first and the second examination. In cortical brain regions with a high density of 5-HT_{2A} receptors, the outcome parameter (BP_1) showed high reproducibility, with a median difference between the two measurements of 6% (range 5–12%). In regions with low receptor density, BP_1 reproducibility was lower, with a median difference of 17% (range 11–39%). A high mean regional BP_1 was associated with low mean variability (R^2=0.67). Reproducibility within the test-retest sample did not differ between PVE-corrected versus non-PVE-corrected data when observing the cortical regions with a high density of receptors. The power analysis calculated from the BP_1 mean and standard deviations from the healthy control sample of 84 subjects (Table 1) showed that in PVE-corrected regions, a sample size of between 24 and 92 subjects is required to detect a 20% difference in BP_1 between two independent groups, with a power of 0.8 and a significance level of 0.05 in a unilateral brain region. Interindividual variability was substantially reduced by applying partial volume correction of data, resulting in a reduction in the required sample sizes (Table 1).

Table 1 Variabilities, intraclass correlations and estimations of sample sizes

Data	Region (left hemisphere)	Test (n=6)			Retest (n=6)			Variability (%)			Sample size (n=84)			Sample size (n=84)		
								Median	PVE	Non-PVE	PVE			Non-PVE		
		Median	Mean	SD	Median	Mean	SD	(95% Cl)	ICC	ICC	Mean	SD	*N*	Mean	SD	*N*
C_p		3.90	4.09	0.58	4.01	4.14	0.50	7 (4–10)	0.79		3.12	1.06		3.12	1.06	
Alt_frac		52.44	52.51	8.71	53.12	53.37	5.43	9 (4–14)	0.71		51.85	7.71		51.85	7.71	
f_1		0.32	0.34	0.10	0.33	0.33	0.08	25 (18–32)	0.57		0.42	0.22		0.42	0.22	
C_p_cor		2.11	2.15	0.50	2.29	2.21	0.33	11 (5–17)	0.80		1.66	0.62		1.66	0.62	
V_d	Cerebellum	2.06	2.09	0.69	2.10	2.14	0.61	12 (5–17)	0.92	0.90	2.11	0.66		1.76	0.59	
BP_1	*High-density regions*															
	Orbitofrontal c.	2.12	2.11	0.37	2.19	2.16	0.39	7 (5–9)	0.91	0.90	2.32	0.60	28	1.30	0.44	46
	Med. inf. frontal c.	3.08	2.85	0.70	2.73	2.72	0.56	5 (0–10)	0.90	0.93	2.93	0.71	24	1.31	0.42	42
	Sup. frontal c.	2.94	2.85	0.66	2.68	2.81	0.60	6 (2–10)	0.92	0.94	2.94	0.71	24	1.22	0.39	43
	Ant. cingulate	2.28	0.62	0.62	2.00	1.93	0.54	12 (2–21)	0.79	0.86	2.12	0.58	30	1.39	0.46	54
	Post. cingulate	2.20	2.19	0.42	2.30	2.22	0.34	6 (2–10)	0.90	0.94	2.36	0.60	27	1.50	0.46	38
	Insula	1.95	1.93	0.50	1.83	1.82	0.44	6 (0–12)	0.89	0.93	1.92	0.51	28	1.43	0.46	44
	Med. inf. temporal c.	2.31	2.20	2.20	2.20	2.15	0.49	9 (0–21)	0.67	0.78	2.43	0.61	27	1.41	0.45	42
	Sup. temp c.	2.43	2.35	0.56	2.45	2.36	0.49	5 (0–11)	0.89	0.94	2.56	0.66	28	1.43	0.46	41
	Sens. motor c.	3.03	2.77	0.62	2.93	2.80	0.64	6 (2–10)	0.93	0.94	2.72	0.70	27	0.96	0.35	55
	Parietal	3.26	3.02	0.65	2.96	3.03	0.62	6 (1–11)	0.91	0.93	3.18	0.76	24	1.27	0.44	50
	Occipital c.	3.01	2.74	0.66	2.88	2.74	0.61	5 (0–10)	0.93	0.93	2.85	0.72	26	1.34	0.43	41
	Low-density regions															
	Entorhinal c.	0.97	0.88	0.31	0.75	0.72	0.37	39 (0–80)	0.02	0.73	0.91	0.44	86	0.36	0.31	309
	Hippocampus	0.51	0.46	0.14	0.51	0.47	0.23	27 (0–60)	0.38	0.51	0.51	0.24	92	0.45	0.24	111
	Thalamus	0.81	0.24	0.24	0.79	0.74	0.11	11 (0–22)	0.44	0.40	0.66	0.25	60	0.31	0.18	143
	Caudate	0.89	0.89	0.28	0.69	0.80	0.20	17 (0–34)	0.38	0.56	0.68	0.30	74	0.22	0.18	205
	Putamen	0.83	0.85	0.19	0.80	0.81	0.06	17 (7–27)	0.14	0.10	0.73	0.27	52	0.54	0.20	64

Parametric measures of volume of distribution (V_d) of cerebellum and regional binding potentials (BP_1) together with variability between test and retest and the intraclass correlation (ICC). For each region, the estimated sample sizes are shown that are required to detect changes with sufficient power between two independent groups. Further, data from 84 subjects were used for calculation of distribution and expected sample size (*N*) to detect regional changes between two independent groups

PVE partial volume estimation corrected, C_p plasma concentration (non-corrected), *Alt_frac* [^{18}F]altanserin fraction, f_1 free fraction of [^{18}F]altanserin, C_p_*cor* metabolite-corrected plasma concentration, *cb* cerebellum

Discussion

This study demonstrates a high reproducibility of BP_1 for 5-HT_{2A} receptor binding as measured with the [^{18}F]altanserin PET bolus/infusion approach. The reproducibility obtained in our bolus/infusion study is generally better than that previously reported for [^{18}F]altanserin bolus/injection data [6]. Our analysis of the individual components constituting BP_1 showed that the non-specific binding, as determined from cerebellar binding, was quite high, whereas the parent compound fraction measurements, as determined with HPLC, reproduced only moderately well, possibly because of day-to-day variations in tracer metabolism. Not surprisingly, because of the low plasma free fraction, the reproducibility of f_1 was relatively low, leading to a large variability and low intraclass correlations for BP ($[V_d(\mathrm{ROI}) - V_d(\mathrm{NS})]/f_1$) for altanserin (these data are not shown). It is important to emphasise that even though every effort was made to ensure standardised experimental conditions, the variability in BP_1 may have derived in part from methodological issues; however, the relative contribution from methodological and biological variability is difficult to assess.

Whereas some effort has been directed towards the question of appropriate design of brain activation studies, much less attention has been given to PET brain neuroreceptor studies in terms of identifying the number of subjects that need to be included in order to detect a statistically significant difference between two groups. In fact, there is reason to believe that many negative PET studies actually may have suffered from type II errors because of lack of power. Unfortunately, only a few other PET studies have dealt with sample size calculations to test the feasibility of the method and, furthermore, they are not directly comparable with our study. For example, Kapur et al. [18] suggested that for an [^{18}F]setoperone PET study to detect a difference greater than 25% in a large cortical brain region with high 5-HT_2 binding, between 12 and 15 subjects would be needed. It should, however, be noted that this calculation was based on larger brain regions in a limited number of control subjects in the same age group.

In spite of excellent reproducibility of [^{18}F]altanserin binding in regions with high receptor density, the required sample size is ultimately determined by the interindividual variability, the power and the significance level (usually 0.05). In these experiments, the power was set to 0.8, which ensures a reasonably high detection of departure from the null hypothesis. It is important to emphasise that in general, power analyses aim at excluding type II errors. That is, statistically significant differences identified in smaller samples may actually be correctly identified. There are several conditions under which a reduction of the sample sizes given in Table 1 would be seen. Inclusion of subjects only within a narrow age range, identification of bilateral differences in brain regions or other approaches likely to minimise the interindividual variation would generally result in a reduction in the sample size. This statement is also illustrated in Table 1, where it is apparent that the standard deviation of the mean is generally larger for the 84 subjects than in the smaller sample of six subjects. This is readily explained by differences between the two groups, the latter being more homogeneous in terms of gender and age. If one could identify one or more factors that influence the interindividual variability, it might be possible to stratify subjects accordingly. Apart from age, such factors have, however, not yet been identified for [^{18}F]altanserin PET studies, though genetic polymorphisms of relevance for the receptor in question may, for example, constitute such a factor.

PVE is a correction of the limited resolution of the PET image resulting in, for example, a significant spillover of counts from grey matter to cerebrospinal fluid. In our study, we observed that the PVE correction was associated with a decrease in the interindividual variability. As expected, PVE correction led to an increase in BP_1 in grey matter (Table 1). The PVE correction improved the precision in the BP_1 determination as reflected in the much smaller sample size resulting from PVE correction. That is, the net outcome of the PVE correction was a decrease in interindividual variability, most likely because the age-associated brain atrophy is better accounted for. The PVE correction did not consistently change reproducibility for any of the brain regions.

Obviously, the low receptor density regions were very sensitive to changes like co-registration, segmentation, automatic delineation and PVE correction. In these regions there is too little signal and too much noise and the grey matter structures have only an extent of a few millimetres in each slice.

In conclusion, the current study supports the feasibility of the [^{18}F]altanserin bolus/infusion approach combined with user-independent delineation of ROIs and PVE correction for determination of 5-HT_{2A} receptor binding in humans. Because of the considerable interindividual variability in receptor binding combined with a lower reproducibility of BP_1 in small brain regions with a low receptor density, a relatively larger sample size is required in studies addressing changes in these regions.

Acknowledgements We wish to thank Karin Stahr and the staff at the PET centre for their technical assistance, and The John and Birthe Meyer Foundation for the donation of the cyclotron and the PET scanner. This work was generously supported by the Danish Health Research Council, The Ludvig and Sara Elsass Foundation, The Hørslev Foundation, Director Jacob Madsen and Wife Olga Madsen's Foundation, The Augustinus Foundation, The Beckett Foundation, The Foundation for Neurological Research, The A.P. Møller and Chastine McKinney Møller Foundation and The Lundbeck Foundation.

This investigation complies with the current laws in Denmark and was approved by the ethics committee in Copenhagen.

References

1. Ito H, Nyberg S, Halldin C, Lundkvist C, Farde L. PET imaging of central 5-HT2A receptors with carbon-11-MDL 100,907. J Nucl Med 1998;39:208–14.
2. Biver F, Goldman S, Luxen A, Monclus M, Forestini M, Mendlewicz J, et al. Multicompartmental study of fluorine-18 altanserin binding to brain 5HT2 receptors in humans using positron emission tomography. Eur J Nucl Med 1994;21:937–46.
3. Kristiansen H, Elfving B, Plenge P, Pinborg LH, Gillings N, Knudsen GM.: Binding characteristics of the 5-HT2A receptor antagonists altanserin and MDL 100907. Synapse 2005;58:249–57.
4. Price JC, Lopresti BJ, Meltzer CC, Smith GS, Mason NS, Huang Y, et al. Analyses of [^{18}F]altanserin bolus injection PET data. II: consideration of radiolabeled metabolites in humans. Synapse 2001;41:11–21.
5. Pinborg LH, Adams KH, Svarer C, Holm S, Hasselbalch SG, Haugbol S, et al. Quantification of 5-HT2A receptors in the human brain using [^{18}F]altanserin-PET and the bolus/infusion approach. J Cereb Blood Flow Metab 2003;23:985–96.
6. Smith GS, Price JC, Lopresti BJ, Huang Y, Simpson N, Holt D, et al. Test-retest variability of serotonin 5-HT2A receptor binding measured with positron emission tomography and [^{18}F]altanserin in the human brain. Synapse 1998;30:380–92.
7. Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S, et al. A database of [^{18}F]-altanserin binding to 5-HT$_{2A}$ receptors in normal volunteers: normative data and relationship to physiological and demographic variables. Neuroimage 2004;21:1105–13.
8. DeGrado TR, Turkington TG, Williams JJ, Stearns CW, Hoffman JM, Coleman RE. Performance characteristics of a whole-body PET scanner. J Nucl Med 1994;35:1398–406.
9. Lewellen TK, Kohlmeyr SG, Miyaoka RS, Kaplan MS, Stearns CW, Schubert SF. Investigation of the performance of the General Electric ADVANCE positron emission tomograph in 3D mode. IEEE Trans Nucl Sci 1996;43:2199–206.
10. Woods RP, Cherry SR., Mazziotta JC. Rapid automated algorithm for aligning and reslicing PET images. J Comput Assist Tomogr 1992;16:620–33.
11. Sadzot B, Lemaire C, Maquet P, Salmon E, Plenevaux A, Degueldre C, et al. Serotonin 5HT2 receptor imaging in the human brain using positron emission tomography and a new radioligand, [^{18}F]altanserin: results in young normal controls. J Cereb Blood Flow Metab 1995;15:787–97.
12. van Dyck CH, Tan PZ, Baldwin RM, Amici LA, Garg PK, Ng CK, et al. PET quantification of 5-HT2A receptors in the human brain: a constant infusion paradigm with [^{18}F]altanserin. J 2000;41:234–41.
13. Lopresti B, Holt D, Mason NS, Huang Y, Ruszkiewicz J, Perevuznik J, et al.: Characterization of the radiolabeled metabolites of [^{18}F]altanserin: implications for kinetic modeling. In: Carson RE, Daube-Witherspoon ME, Herscovitch P, editors. Quantitative functional brain imaging with positron emission tomography. San Diego: Academic Press; 1998; p 293–8.
14. Willendrup P, Pinborg LH, Hasselbalch SG, Adams KH, Stahr K, Knudsen GM, et al. Assessment of the precision in co-registration of structural MR-images and PET-images with localized binding. In: Iida H, Shah NJ, Hayashi T, Watabe H. Quantification in biomedical imaging with PET and MRI. International Congress Series. 2004;1265:275–80.
15. Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, et al. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. Neuroimage 2005;24:969–79.
16. Mueller-Gaertner HW, Links JM, Prince JL, Bryan RN, McVeigh E, Leal JP, et al. Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. J Cereb Blood Flow Metab 1992;12:571–83.
17. Quarantelli M, Berkouk K, Prinster A, Landeau B, Svarer C, Balkay L, et al. Integrated software for the analysis of brain PET/SPECT studies with partial-volume-effect correction. J Nucl Med 2004;45:192–201.
18. Kapur S, Jones C, DaSilva J, Wilson A, Houle S. Reliability of a simple non-invasive method for the evaluation of 5-HT2 receptors using [^{18}F]-setoperone PET imaging. Nucl Med Commun 1997;18:395–9.

Paper II

A serotonin transporter polymorphism influence *in vivo* 5-HT$_{2A}$ binding in the human brain

Steven Haugbøl,[1] Lars H. Pinborg,[1] Vibe G. Frøkjær,[1] David Erritzøe,[1] Lisbeth Marner,[1] Steen G. Hasselbalch,[1] Claus Svarer,[1] Lis Hasholt,[2] Søren Holm,[3] Olaf B. Paulson,[4] Gitte M. Knudsen[1]

[1]Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet and Center for Integrated Molecular Brain Imaging, Denmark.[2]Section of Neurogenetics, Department of Medical Biochemistry and Genetic, University of Copenhagen, Denmark.[3]PET- and Cyclotron Unit, Copenhagen University Hospital, Denmark.[4]Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital, Denmark

Corresponding author:
Steven Haugbøl, MD
Neurobiology Research Unit 9201
The Neuroscience Center
Rigshospitalet, Copenhagen University Hospital
9 Blegdamsvej, DK-2100 Copenhagen, Denmark
Phone: +45 3545 6712, Fax: +45 3545 6713
Email: steven@nru.dk

keywords: *serotonin; 5-HT$_{2A}$ receptor; 5-HT transporter; polymorphisms; VNTR; genetic; PET*

Abstract

The 5-HT$_{2A}$ receptor has together with the serotonin transporter a pivotal role in biological psychiatry. They are implication in several major neuropsychiatric diseases including mood disorders, schizophrenia, obsessive-compulsive disorder and addiction. However, it is unknown which factors regulates the density and activity of the receptor. This study test the influence of two single nucleotide polymorphism in the serotonin 2A receptor gene and two functional polymorphic variable number of tandem repeats in the serotonin transporter gene on *in vivo* serotonin 2A receptor binding in brains of 96 healthy volunteers. For assessment of the binding potential to the 5-HT$_{2A}$ receptor the subjects were investigated with [^{18}F]altanserin PET using a bolus-infusion protocol. The allelic group effects were analyzed with repeated measures ANOVA. The single nucleotide polymorphism 102 *t/c* and 452 *His/Tyr* did not influence [^{18}F]altanserin binding to the 5-HT$_{2A}$ receptor. In contrast, the functional length polymorphism in the second intron of the serotonin transporter gene shows significant effect on the 5-HT$_{2A}$ receptor binding. This effect was increased by adding the effect of the other functional length polymorphism in the promoter of the serotonin transporter. The present results agree with the expanding literature showing that polymorphism in the 5-HTT gene regulate the expression of the 5-HTT. This mechanism is probably an important factor in neurodevelopment since it has effect on the distant but functional related 5-HT$_{2A}$ receptor. This observation intersect with previous studies indicating that these functional polymorphism are associated to personality trait, neuropsychiatric diseases and drug response.

Introduction

The neurotransmitter serotonin (5-HT) plays an important role in mood, anxiety disorders psychosis, personality. 5-HT is synthesized in raphe nuclei located in the brainstem and it distributes widely in CNS to target at least seven different classes of serotonin receptors. The serotonin 2A (5-HT$_{2A}$) receptor is a G-protein coupled receptor and is of particular interest in biological psychiatry because it is a target for treatment of various neuropsychiatric diseases including psychosis, depression, anxiety disorders, and tourette's syndrome (Meltzer, 1999; Scahill et al., 2003; Blier and Szabo, 2005), and, further, it also mediates the action of hallucinogens (Aghajanian and Marek, 1999). Positron emission tomography (PET) studies show increased 5-HT$_{2A}$ receptor binding in depression and obsessive-compulsive disease (Zanardi et al., 2001; Adams et al., 2005) and association between low 5-HT$_{2A}$ binding and harm avoidance in healthy controls (Moresco et al., 2002). Post mortem tissue from depressed (Turecki et al., 1999) and schizophrenic (Hernandez and Sokolov, 2000) patients demonstrate altered mRNA expression of the 5-HT$_{2A}$ receptor. A meta-analysis associates the c allele of the 102 *c/t* single nucleotide polymorphism (SNP) in the 5-HT$_{2A}$ gene (HTR2A) to schizophrenia

(Williams et al., 1996). Furthermore, the amino acid changing SNP 452 *His*/*Tyr* in the coupling zone of the c-terminal region in the HTR2A shows effect on antipsychotic treatment (Yamanouchi et al., 2003). Moreover, serotonin transporter (5-HTT) knock out mice have marked decrease in the cortical density of 5-HT$_{2A}$ receptors (Rioux et al., 1999) which supports that the function of 5-HTT and endogenous serotonin have an influence on the post synaptic 5-HT$_{2A}$ receptor.

In addition, variable-number of tandem repeat (VNTR) polymorphisms in the 5-HTT gene acts as transcriptional regulators (Lesch et al., 1996; MacKenzie and Quinn, 1999). The one in the promoter region (5-HTTLPR) has been linked with vulnerability to affective disorders (Caspi et al., 2003) and different clinical effect of selective serotonin reuptake inhibitors (Malhotra et al., 2004). The second VNTR (VNTR-2) is located in the second intron of the 5-HTT gene has in previous studies been linked to a spectrum of affective disorders (Kunugi et al., 1997; Gutierrez et al., 1998b).

Here we evaluate the role of polymorphisms in HTR2A (-1438 *a*/*g*, 102 *c*/*t*, and 452 *His*/*Tyr*) and 5-HTT gene (5-HTTLPR and VNTR-2) on 5-HT$_{2A}$ receptor binding in healthy humans. We hypothesize that SNP's in the HTR2A or sustained differences in endogenous serotonin levels ascribed to allele variation in 5-HTT gene might change the availability of 5-HT$_{2A}$ receptors in the brain. To our knowledge to date no *in vivo* studies have demonstrated changes of the 5-HT$_{2A}$ expression due to genetic variations.

Material and Methods

Ninety-six healthy individuals (36 women and 60 men) were recruited from newspaper advertising. Median age was 42 years, range 18 to 80 years. All participants were screened and had no history of neurological or psychiatric disease. Likewise they had no alcohol and substance history. On the day of PET scanning they underwent a physical examination by a trained physician. We obtained written informed consent before inclusion. The protocol was approved by the Ethics Committee of Copenhagen and Frederiksberg ((KF) 02-058/99 and (KF) 12-031/02).

A literature search localized three regions with (SNP's) in the 5-HT$_{2A}$ receptor gene (HTR2A; 13q14-q14.3) (Arranz et al., 1995; Arranz et al., 1996; Ohara et al., 1998) and VNTR's in the 5-HTT gene (SLC6A4; 17q11.1-q12) (Lesch et al., 1996; Ogilvie et al., 1996).

Genomic DNA was extracted from full blood and buffy coat lymphocytes using a purification set from Qiagen Incorporate (www.qiagen.com). The HTR2A -1438 *a*/*g* promoter polymorphism was identified by using the PCR protocol described by Masellis et al. (1998) (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'-ATG GCC TTT TGT GCA GAT TC-3' and followed by restriction enzyme digestion with *Msp*I for 4 h. PCR fragment were separated on a 2 % agarose gel (SeaKem GTG Agarose, www.cambrex.com). An *a* at position -1438 lead to an uncut fragment of 200 bp and a *g* at position -1438 lead to two fragments of length 121 bp and 79 bp.

The HTR2A 102 *t*/*c* polymorphism was identified using a PCR protocol described by Quist et al. (2000) (Quist et al., 2000). In short, PCR amplification of a 242 bp fragment was generated by forward primer 5'-TCT GCT ACA AGT TCT GGC TT-3' and reverse primer 5'-CTG CAG CTT TTT CTC TAG GG-3' and followed by restriction enzyme digestion with *Msp*I overnight. PCR fragment were separated on a 2 % agarose gel (SeaKem GTG Agarose, www.cambrex.com). A *t* at position 102 lead to an uncut fragment of 342 base pairs and a *c* at position 102 lead to two fragments of length 216 bp and 132 bp.

The 5-HT$_{2A}$ receptor 452 *His*/*Tyr* polymorphism was identified using a PCR protocol described by Quist et al. (2000) (Quist et al., 2000). In short, PCR amplification of a 155 bp fragment was generated by forward primer 5'-TGA TCG TTG GTT CCA CTA GAC TT-3' and reverse primer 5'-ATA CCG GCT TTG GGC CTA CA-3' and followed by restriction enzyme digestion with *Bsm*I for 4 hours. PCR fragment were separated on a 2 % agarose gel (SeaKem GTG Agarose, www.cambrex.com).The presence of the common *His* allele yielded two fragments of 95 bp and 60 bp while the *Tyr* allele produced one fragment of 155 bp.

The polymorphic VNTR (5-HTTLPR) upstream of the coding region of the 5-HTT gene was identified as described by Mellerup et al. (2001) (Mellerup et al., 2001). In short, PCR amplification of a DNA fragment containing the polymorphic region was generated from the forward primer 5'-GGC GTT GCC GCT CTG AAT TGC-3' and reverse primer 5'-GAG GGA CTG AGC TGG ACA ACC AC-3' and followed by separation on a 2 % agarose (SeaKem GTG Agarose, www.cambrex.com). The presence of the short allele containing 6 copies of the tandem repeat yielded a fragment of 484 bp. The long allele containing 8 tandem repeats produced a fragment of 528 bp.

The VNTR element in intron 2 (VNTR-2) was identified using a PCR protocol described by Mellerup et al. (2001) (Mellerup et al., 2001). In short, PCR amplification of a DNA fragment containing the polymorphic region was generated by forward primer 5'-TGT TCC TAG TCT TAC GCC AGT G-3' and reverse primer 5'-GTC AGT ATC ACA GGC TGC GAG-3' followed by separation on a 3 % agarose gel (NuSieve GTG Agarose, www.cambrex.com) yielded identification of 9, 10 and 12 copies of the tandem repeats.

Subjects were dichotomized as 5-HTTLPR (*s*/*s* + *s*/*l* vs. *l*/*l*) and VNTR-2 (*10*/*10* + *10*/*12* vs. *12*/*12*) consistent with previous studies demonstrating lower transcriptional efficiency of the promoter in short form (s) (Heils et al., 1996) and indications of 10 copies of VNTR-2 has lower expression than 12 copies (Lesch et al., 1994; Fiskerstrand et al., 1999; MacKenzie and Quinn, 1999; Hranilovic et al., 2004). The polymorphism in the HTR2A gene were not dichotomized (102 *t*/*c*: *t*/*t*, *t*/*c*, *c*/*c*; 452 *His*/*Tyr*: *His*/*His*, *His*/*Tyr*, *Tyr*/*Tyr* and -1438 *a*/*g*: *a*/*a*, *a*/*g*, *g*/*g*).

We applied multivariate repeated measures ANOVA (SAS statistical software package SAS 9.0; SAS Institute Inc., Cary, NC, USA) to analyze the effect of the polymorphisms in the HTR2A and SLC6A4 on 5-HT$_{2A}$ receptor binding with [^{18}F]altanserin. Within-subject factors were 16 brain regions genotypes. Between-subject factor were genotype, age, and neuroticism.

PET data acquisition and image analysis procedures have been described in detail previously (Pinborg et al., 2003; Willendrup et al., 2004; Svarer et al., 2005). In short, [^{18}F]altanserin was administrated as a combination of a bolus injection and a continuous infusion (ratio 1.75 hrs) to obtain tracer steady state in blood and tissue. Subjects received a maximal dose of 3.7 MBq/kg bodyweight [^{18}F]altanserin, with an average dose of 270 MBq. PET-scans were performed with an eighteen-ring GE-Advance scanner (General Electric, Milwaukee, Wisconsin, USA). Visual inspection of the time-activity curves was done to assure constant blood and tissue levels. The binding potential (BP_1) was calculated for cortical, limbic and subcortical regions of interest (ROI).

$V_{d(ROI)} = C_{ROI}/C_{plasma}$, $V_{d(NS)} = C_{Cerebellum}/C_{plasma}$ and $BP_1 = [V_{d(ROI)} - V_{d(NS)}]$.
Where $V_{d(ROI)}$ and $V_{d(NS)}$ is volume of distribution of specific binding and non specific binding. C is concentration of radioligand in tissue (ROI/cerebellum) and plasma.

We applied multivariate repeated measures ANOVA (SAS statistical software package SAS 9.0; SAS Institute Inc., Cary, NC, USA) to analyze the effect of the

polymorphisms in the HTR2A and SLC6A4 on 5-HT$_{2A}$ receptor binding. Within subject factors were 16 brain regions (Fig. 1). Between subject factors were age and genotypes.

Results

Results from the genotyping of the healthy Caucasian humans are presented in Table 5. Genotype frequencies of HTR2A (-1438 *a*/*g*, 102 *t*/*c* and 452 *His*/*Tyr*) and SLC6A4 gene polymorphisms (5-HTTLPR and VNTR-2) in the PET group of healthy volunteers all conformed to the Hardy-Weinberg equilibrium. The SNP site -1438 *g*/*a* and 102 *t*/*c* were in perfect disequilibrium so the genotypes were treated as one. The 5-HT$_{2A}$ receptor SNP at site 102 *t*/*c* and 452 *His*/*Tyr* showed no significant effect on [^{18}F]altanserin BP$_1$ values, neither in multivariate repeated measures analysis or single regional binding comparisons. Consequently, they were excluded in the further analysis to reduce noise.

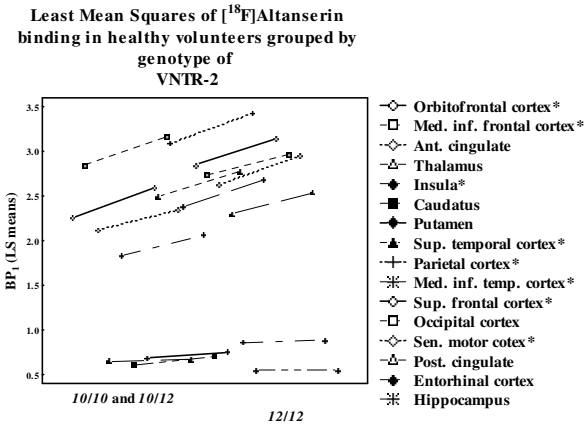
Table 1

Polymorphism	Genotype	Distribution	Percentage	Hardy-Weinberg Chi-square (P value)
102 *t/c*	*t/t*	14	14.6	0.83
	t/c	44	45.8	
	c/c	38	39.6	
452 *h/y*	*h/h*	81	84.4	0.41
	h/y	15	15.6	
	y/y	0	0.0	
5-HTTLPR	*s/s*	19	19.8	0.29
	s/l	53	55.2	
	l/l	24	25.0	
VNTR-2	*12/12*	35	36.5	0.12
	10/12	36	37.5	
	10/10	24	25.0	
	9/9	0	0.0	
	9/10	0	0.0	
	9/12	1	1.0	

Group characteristics and genotype frequencies for 5-HT$_{2A}$ and 5-HTT polymorphisms in the investigated population of healthy Caucasian volunteers. 102 t/c and 452 *His*/*Tyr* are polymorphisms in the 5-HT$_{2A}$ receptor gene. 5-HTTLPR and VNTR-2 are length polymorphisms in the 5-HTT gene.

Multivariate repeated measures ANOVA of the group allelic effect of VNTR-2 on 5-HT$_{2A}$ receptor binding were significant, F = 4.18, P = 0.04, when adjusting for age. By adding 5-HTTLPR as a covariate to the model the allelic effect of VNTR-2 got more significant (F = 6.00, P = 0.02). The allelic effect of 5-HTTLPR were not significant (F = 2.95, P = 0.09). In general, BP$_1$ values of VNTR-2 *10*/*10* and *10*/*12* were universally lower in all tested brain regions than subjects carrying *12*/*12* (Fig. 1).

Figure 1



Post hoc analyses. General linear model where mean regional BP_1 of [^{18}F]altanserin are dependent variables and VNTR-2 polymorphism in the 5-HTT gene and age are independent variables. VNTR-2 polymorphism is dichotomized: 10/10 + 10/12 (left points) or 12/12 (right points). *P <0.05 indicates significant group difference.

Discussion

In this study we observe a group allelic effect of VNTR-2 which reduces 5-HT$_{2A}$ receptor binding in individuals carrying the 10 allele. These findings provide supports for evolutionary mechanisms that affect the transcriptions for thereby generating polymorph regulatory elements. Thereby, it connects to the hypothesis, which inspired this study, that differential expression of the 5-HTT underlie regulation of serotonergic neurotransmission (Heils et al., 1996; Fiskerstrand et al., 1999; Lovejoy et al., 2003) and as a consequence the regulation of the distinct but connected 5-HT$_{2A}$ receptor.

The results were influence by effect from the 5-HTTLPR, which further argues that the transcriptional promoter element act in concert with the regulatory elements in second intron, as suggested in previous studies (Gutierrez et al., 1998a; Soeby et al., 2005). The 5-HTT protein works by sodium dependent reuptake of 5-HT into the presynapse, but the basis of this activity is poorly understood. Mechanistically, we imagine that low expression of 5-HTT due to the *s* and *10* allele leads to lifelong increase in endogenous 5-HT tone and downregulating of 5-HT$_{2A}$ receptors. Previous studies and scientific reviews supports that regulations of the 5-HT$_{2A}$ receptor may be a natural mechanism to adjust serotonergic transmission in response to physiological demands (Buckholtz et al., 1988; Van Oekelen et al., 2003).

Other in vivo imaging studies ties to this observation. One study reports higher 5-HTT availability in the raphe area in healthy volunteers homozygous for the long insertion *l/l* (Heinz et al., 2000) which connects to higher transcription activity for the *l* allele and another study show that carriers of the *s* allele of 5-HTTLPR reduce 5-HT$_{1A}$ receptor availability in humans (David et al., 2005).

In contrast, we did not find any effect of the 5-HT$_{2A}$ receptor gene 102 *t/c* and 452 *His/Tyr* which for 452 *His/Tyr* could be due to a low frequency of the *Tyr* allele. However for 102 *t/c* it agrees with findings that the SNP not affecting mRNA levels in the brain (Bray et al., 2004) but it is in contrast to the c allele being expressed 20% lower

than the t allele (Polesskaya and Sokolov, 2002). Our results strongly suggest an absence of polymorph factors in the HTR2A causing variability in transcription activity or binding potential of the 5-HT$_{2A}$ receptor. As a consequence, our data allow us to questioning if pathological associations to the 102 *t/c* locus can be explained by direct effects of mRNA expression or conformational alterations in the binding pocket of the 5-HT$_{2A}$ receptor protein. However, it is theoretically possible that our findings may not be relevant to the developmental nervous system since we investigated binding in only healthy adult brains, and that pathological changes could only be attributable in the immature brain.

It is our conviction that the observed widespread homogenous allelic effect of the 5-HTTLPR disadvantages that the results are incidental finding. The post hoc analysis showed that BP_1 was lower by 5-40% in cortical regions. The difference being largest between individuals with both the *s* and *10* allele versus individuals homozygote for both *12/12* and *l/l* allele (data not shown).

As a term of reference, our recent test retest variability of this PET method was 5-12% in cortical regions (Haugbøl et al., 2006), thus, the observed changes appear to be robust findings. Moreover, a PET study with 96 subjects is in large scale numerically compared with other recent neuroimaging studies showing plausible associations.

Taken together, the polymorphic effect on the 5-HT$_{2A}$ receptor binding has consequences for our understanding of the serotonin system. Congenital 5-HTT functioning may be critical in the mammalian brain development. since perturbation of 5-HHT levels during neonatal period has shown disrupt in cortical development (Lesch, 2001; Gaspar et al., 2003). Though, reduced 5-HT$_{2A}$ receptor binding could underlie functional impairment in cortico-striatale-thalomo cortical loops from the neonatal period. Thereby supporting the hypothesis that that the genetic makeup can predispose to mental disorders either congenital or under certain life events (Caspi et al., 2003).

In conclusion, these finding suggest that abnormally high cortical 5-HT levels due to inferior working 5-HTT may underlie a reduced density of the postsynaptic 5-HT$_{2A}$ receptor. Our findings are to our knowledge the first human study to indicate effects of genetic variations in the 5-HTT on the functionally related but distinct 5-HT$_{2A}$ receptor. This result will influence future [^{18}F]altanserin-PET investigations by controlling for genotype and thereby constrain the interindividual variability. Our results complement the expanding literature covering link between 5-HTT and behavioral traits, diseases and pharmacogenetics.

References

Adams KH, Hansen ES, Pinborg LH, Hasselbalch SG, Svarer C, Holm S, Bolwig TG, Knudsen GM (2005) Patients with obsessive-compulsive disorder have increased 5-HT2A receptor binding in the caudate nuclei. Int J Neuropsychopharmacol 8:391-401.

Aghajanian GK, Marek GJ (1999) Serotonin and hallucinogens. Neuropsychopharmacology 21:16S-23S.

Arranz M, Collier D, Sodhi M, Ball D, Roberts G, Price J, Sham P, Kerwin R (1995) Association between clozapine response and allelic variation in 5-HT2A receptor gene. Lancet 346:281-282.

Arranz MJ, Collier DA, Munro J, Sham P, Kirov G, Sodhi M, Roberts G, Price J, Kerwin RW (1996) Analysis of a structural polymorphism in the 5-HT2A receptor and clinical response to clozapine. Neurosci Lett 217:177-178.

Blier P, Szabo ST (2005) Potential mechanisms of action of atypical antipsychotic medications in treatment-resistant depression and anxiety. J Clin Psychiatry 66 Suppl 8:30-40.

Bray NJ, Buckland PR, Hall H, Owen MJ, O'Donovan MC (2004) The serotonin-2A receptor gene locus does not contain common polymorphism affecting mRNA levels in adult brain. Mol Psychiatry 9:109-114.

Buckholtz NS, Zhou DF, Freedman DX (1988) Serotonin2 agonist administration down-regulates rat brain serotonin2 receptors. Life Sci 42:2439-2445.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301:386-389.

David SP, Murthy NV, Rabiner EA, Munafo MR, Johnstone EC, Jacob R, Walton RT, Grasby PM (2005) A functional genetic variation of the serotonin (5-HT) transporter affects 5-HT1A receptor binding in humans. J Neurosci 25:2586-2590.

Fiskerstrand CE, Lovejoy EA, Quinn JP (1999) An intronic polymorphic domain often associated with susceptibility to affective disorders has allele dependent differential enhancer activity in embryonic stem cells. FEBS Lett 458:171-174.

Gaspar P, Cases O, Maroteaux L (2003) THE DEVELOPMENTAL ROLE OF SEROTONIN: NEWS FROM MOUSE MOLECULAR GENETICS. Nature Reviews Neuroscience 4:1002-1012.

Gutierrez B, Pintor L, Gasto C, Rosa A, Bertranpetit J, Vieta E, Fananas L (1998a) Variability in the serotonin transporter gene and increased risk for major depression with melancholia. Hum Genet 103:319-322.

Gutierrez B, Arranz MJ, Collier DA, Valles V, Guillamat R, Bertranpetit J, Murray RM, Fanas L (1998b) Serotonin transporter gene and risk for bipolar affective disorder: an association study in Spanish population. Biol Psychiatry 43:843-847.

Haugbøl S, Pinborg LH, Haroon AM, Frøkjær V, Madsen J, Dyrby TB, Svarer C, Knudsen GM (2006) Reproducibility and sample size estimation of 5-HT2A receptor measurements with [18F]altanserin PET bolus plus infusion approach. European Journal of Nuclear Medicine and Molecular Imaging (Accepted Sep 21 2006).

Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP (1996) Allelic variation of human serotonin transporter gene expression. J Neurochem 66:2621-2624.

Heinz A, Jones DW, Mazzanti C, Goldman D, Ragan P, Hommer D, Linnoila M, Weinberger DR (2000) A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. Biol Psychiatry 47:643-649.

Hernandez I, Sokolov BP (2000) Abnormalities in 5-HT2A receptor mRNA expression in frontal cortex of chronic elderly schizophrenics with varying histories of neuroleptic treatment. J Neurosci Res 59:218-225.

Hranilovic D, Stefulj J, Schwab S, Borrmann-Hassenbach M, Albus M, Jernej B, Wildenauer D (2004) Serotonin transporter promoter and intron 2 polymorphisms: relationship between allelic variants and gene expression. Biol Psychiatry 55:1090-1094.

Kunugi H, Hattori M, Kato T, Tatsumi M, Sakai T, Sasaki T, Hirose T, Nanko S (1997) Serotonin transporter gene polymorphisms: ethnic difference and possible association with bipolar affective disorder. Mol Psychiatry 2:457-462.

Lesch KP (2001) Variation of serotonergic gene expression: neurodevelopment and the complexity of response to psychopharmacologic drugs. Eur Neuropsychopharmacol 11:457-474.

Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, Riederer P (1994) Organization of the human serotonin transporter gene. J Neural Transm Gen Sect 95:157-162.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274:1527-1531.

Lovejoy EA, Scott AC, Fiskerstrand CE, Bubb VJ, Quinn JP (2003) The serotonin transporter intronic VNTR enhancer correlated with a predisposition to affective disorders has distinct regulatory elements within the domain based on the primary DNA sequence of the repeat unit. Eur J Neurosci 17:417-420.

MacKenzie A, Quinn J (1999) A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. Proc Natl Acad Sci U S A 96:15251-15255.

Malhotra AK, Murphy GM, Jr., Kennedy JL (2004) Pharmacogenetics of psychotropic drug response. Am J Psychiatry 161:780-796.

Masellis M, Basile V, Meltzer HY, Lieberman JA, Sevy S, Macciardi FM, Cola P, Howard A, Badri F, Nothen MM, Kalow W, Kennedy JL (1998) Serotonin subtype 2 receptor genes and clinical response to clozapine in schizophrenia patients. Neuropsychopharmacology 19:123-132.

Mellerup E, Bennike B, Bolwig T, Dam H, Hasholt L, Jorgensen MB, Plenge P, Sorensen SA (2001) Platelet serotonin transporters and the transporter gene in control subjects, unipolar patients and bipolar patients. Acta Psychiatr Scand 103:229-233.

Meltzer HY (1999) The role of serotonin in antipsychotic drug action. Neuropsychopharmacology 21:106S-115S.

Moresco FM, Dieci M, Vita A, Messa C, Gobbo C, Galli L, Rizzo G, Panzacchi A, De Peri L, Invernizzi G, Fazio F (2002) In vivo serotonin 5HT(2A) receptor binding and personality traits in healthy subjects: a positron emission tomography study. Neuroimage 17:1470-1478.

Ogilvie AD, Battersby S, Bubb VJ, Fink G, Harmar AJ, Goodwim GM, Smith CA (1996) Polymorphism in serotonin transporter gene associated with susceptibility to major depression. Lancet 347:731-733.

Ohara K, Nagai M, Tsukamoto T, Tani K, Suzuki Y, Ohara K (1998) 5-HT2A receptor gene promoter polymorphism--1438G/A and mood disorders. Neuroreport 9:1139-1141.

Pinborg LH, Adams KH, Svarer C, Holm S, Hasselbalch SG, Haugbol S, Madsen J, Knudsen GM (2003) Quantification of 5-HT2A receptors in the human brain using [18F]altanserin-PET and the bolus/infusion approach. J Cereb Blood Flow Metab 23:985-996.

Polesskaya OO, Sokolov BP (2002) Differential expression of the "C" and "T" alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. J Neurosci Res 67:812-822.

Quist JF, Barr CL, Schachar R, Roberts W, Malone M, Tannock R, Basile VS, Beitchman J, Kennedy JL (2000) Evidence for the serotonin HTR2A receptor gene as a susceptibility factor in attention deficit hyperactivity disorder (ADHD). Mol Psychiatry 5:537-541.

Rioux A, Fabre V, Lesch KP, Moessner R, Murphy DL, Lanfumey L, Hamon M, Martres MP (1999) Adaptive changes of serotonin 5-HT2A receptors in mice lacking the serotonin transporter. Neurosci Lett 262:113-116.

Scahill L, Leckman JF, Schultz RT, Katsovich L, Peterson BS (2003) A placebo-controlled trial of risperidone in Tourette syndrome. Neurology 60:1130-1135.

Soeby K, Larsen SA, Olsen L, Rasmussen HB, Werge T (2005) Serotonin transporter: evolution and impact of polymorphic transcriptional regulation. Am J Med Genet B Neuropsychiatr Genet 136:53-57.

Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, Holm S, Paulson OB, Knudsen GM (2005) MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. Neuroimage 24:969-979.

Turecki G, Briere R, Dewar K, Antonetti T, Lesage AD, Seguin M, Chawky N, Vanier C, Alda M, Joober R, Benkelfat C, Rouleau GA (1999) Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide. Am J Psychiatry 156:1456-1458.

Van Oekelen D, Luyten WH, Leysen JE (2003) 5-HT2A and 5-HT2C receptors and their atypical regulation properties. Life Sci 72:2429-2449.

Willendrup P, Pinborg LH, Hasselbalch SG, Adams KH, Stahr K, Knudsen GM, Svarer C (2004) Assessment of the precision in co-registration of structural MR-images and PET-images with localized binding. In: Iida H. Shah NJ. Hayashi T. and Watabe H. Quantification in Biomedical Imaging with PET and MRI. International Congress Series. 1265:275-280.

Williams J, Spurlock G, McGuffin P, Mallet J, Nothen MM, Gill M, Aschauer H, Nylander PO, Macciardi F, Owen MJ (1996) Association between schizophrenia and T102C polymorphism of the 5-hydroxytryptamine type 2a-receptor gene. European Multicentre Association Study of Schizophrenia (EMASS) Group. Lancet 347:1294-1296.

Yamanouchi Y, Iwata N, Suzuki T, Kitajima T, Ikeda M, Ozaki N (2003) Effect of DRD2, 5-HT2A, and COMT genes on antipsychotic response to risperidone. Pharmacogenomics J 3:356-361.

Zanardi R, Artigas F, Moresco R, Colombo C, Messa C, Gobbo C, Smeraldi E, Fazio F (2001) Increased 5-hydroxytryptamine-2 receptor binding in the frontal cortex of depressed patients responding to paroxetine treatment: a positron emission tomography scan study. J 21. 21:53-58, 53-58.

Paper III

International Journal of Neuropsychopharmacology, Page 1 of 8. Copyright © 2006 CINP
doi:10.1017/S1461145706006559

ARTICLE

Cerebral 5-HT_{2A} receptor binding is increased in patients with Tourette's syndrome

Steven Haugbøl[1], Lars H. Pinborg[1], Lisbeth Regeur[2], Elsebet S. Hansen[3], Tom G. Bolwig[3], Finn Å. Nielsen[1], Claus Svarer[1], Lene T. Skovgaard[4] and Gitte M. Knudsen[1]

[1] *Neurobiology Research Unit, Copenhagen University Hospital, Denmark*
[2] *Department of Neurology, Copenhagen University Hospital, Denmark*
[3] *Department of Psychiatry, Copenhagen University Hospital, Denmark*
[4] *Department of Biostatistics, University of Copenhagen, Denmark*

Abstract

Experimental and clinical data have suggested that abnormalities in the serotonergic neurotransmissions in frontal-subcortical circuits are involved in Tourette's syndrome. To test the hypothesis that the brain's 5-HT_{2A} receptor binding is increased in patients with Tourette's syndrome, PET imaging was performed. Twenty adults with Tourette's syndrome and 20 healthy control subjects were investigated with PET-[^{18}F]altanserin using a bolus-infusion protocol. Regions of interest were delineated automatically on co-registered MRI images, and partial volume-corrected binding parameters were extracted from the PET images. Comparison between control subjects and Tourette's syndrome patients showed increased specific [^{18}F]altanserin binding, not only in the a-priori selected brain regions hypothesized to be involved in Tourette's syndrome, but also post-hoc analysis showed a global up-regulation when testing for a overall difference with a randomization test ($p < 0.03$). Increased 5-HT_{2A} receptor binding was found not only in regions closely related to subcortical regions in patients with Tourette's syndrome, but also in most other brain regions. Our data suggest that the serotonergic transmitter system is pathophysiologically involved in Tourette's syndrome and that a clinical trial with 5-HT_{2A} receptor antagonists may be justified.

Received 4 October 2005; Reviewed 8 November 2005; Revised 6 January 2006; Accepted 12 January 2006

Key words: Altanserin, automatic region delineation, bolus infusion, partial volume correction, PET, serotonin receptor, tics, Tourette's syndrome.

Introduction

Tourette's syndrome (TS) is a neurological disorder that commences in childhood and is characterized by motor and vocal tics (Robertson, 2000). The tics can largely be suppressed by treatment with D_2 antagonist (Regeur et al., 1986) and in-vivo studies have demonstrated abnormalities of striatal dopaminergic neurotransmission (Cheon et al., 2004; Singer et al., 2002), although the results have been somewhat conflicting (Stamenkovic et al., 2001; Turjanski et al., 1994). The clinical picture of TS has a comorbidity with other disorders such as obsessive–compulsive disorder (OCD) (Comings and Comings, 1987a), mood disorders (Comings and Comings, 1987b) and migraine (Kwak et al., 2003), where disturbances of the serotonergic transmitter system are involved. Neurobiological studies also suggest a central serotonin (5-HT) dysfunction in TS. The 5-HT precursor, tryptophan, is decreased in plasma (Comings, 1990) and reduced tryptophan, 5-HT and its metabolite 5-HIAA in subcortical and cortical brain regions has also been reported (Anderson et al., 1992). Further, two SPECT studies with [^{123}I]β-CIT have shown changes of the of midbrain 5-HT transporter density in drug-free TS patients (Heinz et al., 1998; Muller-Vahl et al., 2005). In particular, the 5-HT_{2A} receptor is likely to be involved in motor control since 5-HT_{2A} receptor agonist treatment in rodents induces hyperlocomotion (Bishop et al., 2004) and motor behaviour and hyperlocomotion are attenuated by 5-HT_{2A} receptor antagonism (Higgins et al., 2003; Ninan and Kulkarni, 1998). In patients with TS, risperidone, an atypical antipsychotic drug with affinity for the 5-HT_{2A} receptor is an effective treatment for tics

Address for correspondence: S. Haugbøl, M.D., Neurobiology Research Unit 9201, The Neuroscience Center, Copenhagen University Hospital, 9 Blegdamsvej DK-2100 Copenhagen, Denmark.
Tel.: +45 35456706 *Fax*: +45 35456713
E-mail: steven@nru.dk

(Scahill et al., 2003), and ziprasidone and olanzapine, also atypical antipsychotics with high affinity for the 5-HT_{2A} receptor, have in case reports also showed promising treatment effect. In addition, a small open clinical study has indicated that ketanserin, a compound with mainly 5-HT_{2A} antagonist properties, is effective for treating tics (Bonnier et al., 1999).

Abnormal function of loop circuits interconnecting the basal ganglia and frontal cortex (motor planning, affect, behavioural inhibition) and limbic areas (motivational and threat detection) are thought to be critically involved in TS (Leckman, 2002). It is believed that many cortical regions project to the striosomal part of striatum and play a role in motor stereotypes (Saka and Graybiel, 2003). Imaging studies have previously identified changes in frontal, paralimbic and striatal areas in TS, both in studies of glucose metabolism (Braun et al., 1993) and in activation studies with magnetic resonance imaging (MRI) (Peterson et al., 1998) and positron emission tomography (PET) (Stern et al., 2000). In this study we PET scanned TS patients and age- and gender-matched healthy control subjects with the selective 5-HT_{2A} receptor antagonist [^{18}F]altanserin to investigate regions known to be particularly involved in TS: anterior cingulate, prefrontal cortex, orbitofrontal cortex, putamen, and caudate.

Methods

Subjects

Patients with a diagnosis of TS (8 women and 12 men) were recruited from the TS clinic at Copenhagen University Hospital. Median age was 26 yr (range 17–49 yr). The diagnosis was established by an experienced neurologist, based on DSM-IV criteria (APA, 1994). Only patients with clearly visible tics and TS as their primary disorder were included. Patients with intrusive obsessive or compulsive symptoms were excluded. Likewise, patients with mood disorders and other psychiatric disorders, including anxiety, were also excluded. The protocol was approved by the Ethics Committee of Copenhagen and Frederiksberg City [(KF) 02-058/99 and (KF) 12-031/02]. Written informed consent was obtained before inclusion. Tic intensity was rated with the Yale Global Tic Severity Scale (YGTSS) at the visit for clinical examination and at the same day as the PET scanning. On the day of PET scanning the patients were assessed with the Hamilton Rating Scale for Depression (HAMD), Yale–Brown Obsessive–Compulsive Scale (YBOCS) and Brief Psychiatric Rating Scale (BPRS) by a trained psychiatrist. On the scanning day, the patients filled out a self report questionnaire, the system inventory (SCL-90-R) which reflects nine different symptom dimensions.

Table 1. Medication dosage and time schedule in 10 Tourette's syndrome patients

No.	Age	Gender	Medication
1	32	F	Citalopram 40 mg × 1
2	23	F	Pimozide 1 mg × 2
3	22	M	Pimozide 1 mg × 2
4	28	M	Lamotrigene 200 mg × 2
5	23	M	Pimozide 1 mg × 1
6	44	F	Pimozide 1 mg × 3, citalopram 40 mg × 1
7	24	M	Sertralin 100 mg × 1
8	26	F	Pimozide 2 mg × 2, tetrabenazine 25 mg × 2
9	18	F	Pimozide 1 mg × 3
10	17	M	Pimozide 1 mg × 2

Patients taking citalopram and sertralin abstained from treatment 3–6 wk before PET scanning.

None of the patients were drug naive, 10 subjects were medication free for at least 9 months prior to this investigation and 10 TS patients were using drugs to control tics or behaviour (for details see Table 1). The three subjects on selective serotonin reuptake inhibitor (SSRI) treatment abstained from treatment 3–6 wk prior to the PET scan. Twenty age- and gender-matched control subjects were recruited by newspaper advertisement. They had no history of neurological or psychiatric disease and since a normal neurological examination was found on the day of PET scanning, testing with YGTSS, YBOCS, HAMD or BPRS was not conducted. Each of the controls was selected to closely match a TS patient. Median age of controls was 28 yr (range 18–49 yr).

MRI

MRI was performed on a 1.5 T Vision scanner (Siemens, Erlangen, Germany) where 158 slices were acquired continuously in the sagittal plane using a 3D MPRAGE sequence (TE = 4 ms, TR = 11 ms, TI = 300 ms, flip angle = 12°, image matrix = 256 × 256, slice thickness = 1.14 mm, in-plane resolution 1.21 × 1.21 mm).

[^{18}F]Altanserin PET imaging

[^{18}F]Altanserin PET scanning was conducted as described by Pinborg et al. (2003). [^{18}F]Altanserin

was administrated as a combination of a bolus injection and a continuous infusion (ratio 1.75 h) to obtain a tracer steady state in blood and tissue. Subjects received a maximum dose of 3.7 MBq/kg bodyweight [^{18}F]altanserin, with an average dose of 270 MBq. PET scans were performed with an 18-ring GE Advance scanner (General Electric, Milwaukee, WI, USA) operating in 3D-acquisition mode, producing 35 image slices with an interslice distance of 4.25 mm. The technical specifications have been described elsewhere (DeGrado et al., 1994; Lewellen et al., 1996). Dynamic 3D emission scans (five frames of 8 min each) started 120 min after administration of [^{18}F]altanserin. The five dynamic frames were aligned and resliced using the approach from Woods et al. (1992) and subsequently an average image was generated. For all subjects, blood was sampled manually at mid-PET frame-times and analysed with HPLC for determination of the activity of the non-metabolized tracer in plasma (free fraction, f_1).

Determination of parent compound in plasma

The plasma metabolites of [^{18}F]altanserin were determined using a modification of previously published procedures (Lopresti et al., 1998; Sadzot et al., 1995; van Dyck et al., 2000). Plasma whole-blood samples (13 ml) were centrifuged for 10 min at 4700 *g*. Plasma (0.5 ml) was counted in a gamma counter (Cobra 5003, Packard Instruments, Meriden, CT, USA) for determination of radioactivity. Five millilitres of plasma was mixed with acetic acid (20 ml, 50 mmol/l) and eluted through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA, USA), which had been preactivated with methanol (2 ml) and water (10 ml). The Sep-Pak was washed with triethylamine (10 ml, 0.1%) and water (10 ml) before the outlet was connected to a filter (Millipore 0.45 μm), and the radioactive content was eluted with methanol (1 ml) followed by distilled water (3 ml). The resulting mixture (4 ml) was injected on the semi-preparative HPLC system [column: Waters μBondapack C18, 7.8 × 300 mm; solvent A: CH_3COONH_4 (0.073 mol/l, adjusted to pH 4.5 with CH_3COOH); solvent B: 70% CH_3CN/30% CH_3COONH_4 (0.22 mol/l); flow: 6 ml/min)] equipped with an ultraviolet detector (254 nm) and a Packard flow scintillation analyser (500TR series) connected in series. Solvent B was increased with a linear gradient from 25% to 100% for 10 min. The [^{18}F]altanserin eluted after 8.4 min and was well separated from the metabolites eluting after 5.1, 5.6, and 7.3 min respectively.

MR/PET co-registration

MRI images were co-registered using a Matlab-based interactive program (Mathworks Inc., Natick, MA, USA) which is based on visual identification of the transformation (Willendrup et al., 2004). MRIs were segmented into grey matter, white matter, and cerebrospinal fluid (CSF) tissue classes using overlay of prior probability images (SPM2). A total of 35 regions of interest (ROI) were automatically delineated on MRI slices and transferred to PET images using the identified rigid-body transformation (Svarer et al., 2005). PET images were partial volume-corrected using the segmented MRI. A three-compartment model based on grey matter, white matter, and CSF tissue was used (Mueller-Gaertner et al., 1992; Quarantelli et al., 2004). The white-matter value was extracted as the average voxel value from a white-matter ROI (midbrain) in the uncorrected PET image.

The mean count density within each ROI was converted to kBq/cc. The individual cerebellum values, representing non-specific binding only (Pinborg et al., 2003) and decay-corrected plasma [^{18}F]altanserin curves were used to calculate the binding potential (BP_1) values for each ROI according to:

$$BP_1 = \frac{C_{ROI} - C_{ref}}{C_{plasma}} = f_1 \cdot \frac{B'_{max}}{K_d} \quad (\text{ml ml}^{-1}), \tag{1}$$

where C_{ROI} and C_{ref} are mean activities in the volume of interest and in the reference region respectively, C_{plasma} is the activity of non-metabolized tracer in plasma, f_1 is the free fraction of radiotracer, B'_{max} is the density of available receptor sites, and K_d is the affinity of the receptor for the radiotracer.

Statistical analysis

We applied PROC GLM (statistical software package SAS 9.0; SAS Institute Inc., Cary, NC, USA) to analyse data. PROC GLM implements the General Linear Model and was used for the analysis of BP_1 in regions as a dependent variable and age as a quantitative covariate and condition as categorical covariate ($BP_{1,region}$ = age condition). p values for the t statistics tell us whether the variable *condition* has statistically significant predictive capability in the presence of the variable *age*. In addition we performed a test for a general difference of BP_1 between controls and patients. Regional means were averaged across subjects for each condition and we used PROC GLM with age and condition as covariates as described above. However, this relies on the normal distribution assumption, so for robustness considerations, we performed a randomization test

(100 000 random group allocations) to evaluate the test statistic for condition (Edgington, 1995).

Results

For TS patients the median YGTSS score for motor tics was 19 (range 12–23) and the median score for phonetic tics was 11 (range 0–17). In TS patients, the median YBOCS score for obsessions was 4 (range 0–13) and for compulsions 5 (range 0–13). Seven patients had an YBOCS score >10. Two out of these seven patients were among those previously treated with SSRIs. The median HAMD score was 2 (range 0–11) and median BPRS score was 3 (range 1–8). The SCL-90-R scores revealed that the TS patients' median score for anxious personality components of 15 (range 12–28) was significantly higher than the control subjects' score of 11 (range 10–19). This difference was mainly caused by two questions: 'Feeling tense or keyed up' and 'Feeling so restless you couldn't sit still'. Both questions can be considered as coining key features of TS patients. In patients with TS [^{18}F]altanserin binding was significantly increased in the brain regions hypothesized to be involved in the disease (Table 2). In addition, a post-hoc analysis identified a global difference between controls and patients with a higher BP_1 in TS patients ($p < 0.03$). A separate analysis of the 10 drug-free patients also showed a statistically significant increase in 5-HT_{2A} receptor binding compared to their age-matched controls. There was no statistically significant difference in BP_1 between TS patients with a YBOCS score under and over 10 or between drug-free and drug-treated patients. Further, a comparison of pimozide-treated TS patients ($n = 7$) with the rest of the patients ($n = 13$) and healthy control subjects ($n = 20$) was done and revealed no differences in terms of cerebellar uptake, plasma concentration, parent compound of tracer, or binding potentials. In addition, the pimozide-treated patients had the same clinical scores as the rest of the patients. A regression analysis did not show any significant relations between age-adjusted BP_1 and tic severity or YBOCS score, when stratification for drug use was done. Regression analyses associating BP_1 with tic severity done for the combined and separate scores for motor and vocal tics, as well as for obsessions and compulsions did not reveal any significant associations.

Finally, we did not find any systematic changes between TS patients and control subjects in parameters that could have biased the results, e.g. non-specific binding in the cerebellum [1.81 ± 0.34 (mean $\pm$ S.D.) vs. 1.80 ± 0.28], non-protein bound fraction (f_1) (0.38% $\pm$0.11 vs. 0.42% $\pm$0.18), fraction of [^{18}F]altanserin in parent compound (51.6% $\pm$10.3 vs. 53.67% $\pm$8.0) and time from bolus injection to scan start.

Discussion

This is the first PET study to evaluate cerebral 5-HT_{2A} receptor binding in TS patients. The 5-HT_{2A} receptor binding was not only significantly increased in most brain regions hypothesized to be involved in TS patients, but a difference was also found in a large number of other brain regions (Table 2). Overall, [^{18}F]altanserin seems to be globally increased in patients with TS and differences in statistical significance between investigated brain regions presumably reflects differences in variance.

In PET studies involving only one scanning it is usually assumed that radiotracer affinity is the same between groups and that any differences in radiotracer binding reflects differences in B'_{max}. The increase in 5-HT_{2A} receptor binding found in the present study must mean that either TS patients have an increase in the density of available 5-HT_{2A} receptor-binding sites (B'_{max}), an increased affinity of [^{18}F]altanserin to the 5-HT_{2A} receptor, or a combination [eqn (1)]. Our finding of a global increase in 5-HT_{2A} binding may result from conformational changes in the receptor associated with a higher affinity of the 5-HT_{2A} receptor. We wish to emphasize, however, that both an increased B'_{max} and an increased 5-HT affinity could result in an augmented stimulation of the 5-HT_{2A} receptor. Such stimulation from frontal regions may activate the striosomes (either directly or via dopamine) and induce urges or motor tics (Graybiel and Canales, 2001; Saka and Graybiel, 2003). This is supported by several studies in both humans and rodents where global agonist activation of the 5-HT_{2A} receptor with DOI [(±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane], fenfluramine and psilocybin have been shown to increase synaptic dopamine levels in the brain striatum (Kuroki et al., 2003; Smith et al., 1997; Vollenweider et al., 1999). It has previously been reported that TS patients have decreased D_2-receptor binding after challenge with amphetamine which is consistent with the notion that dopamine levels are increased in these patients (Singer et al., 2002). Our data could suggest that an increased striatal dopamine release in TS patients is brought about by overstimulation of 5-HT_{2A} receptors, but based on our data, we cannot conclude if the changes in 5-HT_{2A} receptors are primary or secondary to other alterations. For example, the increase of 5-HT_{2A} receptor binding could be a relevant compensatory

Table 2. Regional BP_1 values

	Control subjects		Tourette patients		
Region of interest	Mean	S.D.	Mean	S.D.	*p* value
A-priori selected regions					
Ant. cingulate, left	2.20	0.57	2.65	0.43	0.005
Ant. cingulate, right	2.29	0.55	2.85	0.40	0.002
Orbitofrontal cortex, left	2.45	0.58	2.92	0.48	0.008
Orbitofrontal cortex, right	2.46	0.66	2.97	0.54	0.01
Med. inf. frontal cortex, left	3.08	0.70	3.46	0.60	0.07
Med. inf. frontal cortex, right	3.14	0.70	3.65	0.64	0.07
Sup. frontal cortex, left	3.18	0.67	3.66	0.68	0.03
Sup. frontal cortex, right	3.21	0.69	3.71	0.70	0.001
Caudate, left	0.69	0.19	0.75	0.23	0.41
Caudate, right	0.56	0.19	0.60	0.19	0.83
Putamen, left	0.66	0.15	0.75	0.17	0.07
Putamen, right	0.62	0.17	0.67	0.24	0.06
Post-hoc analysis					
Thalamus, left	0.68	0.20	0.74	0.22	0.43
Thalamus, right	0.65	0.20	0.78	0.22	0.06
Insula, left	2.12	0.56	2.37	0.40	0.1
Insula, right	2.03	0.44	2.36	0.38	0.003
Sup. temp cortex, left	2.67	0.65	3.02	0.39	0.04
Sup. temp cortex, right	2.76	0.61	3.08	0.46	0.003
Parietal, left	3.29	0.67	3.84	0.59	0.009
Parietal, right	3.32	0.67	3.91	0.59	0.007
Med. inf. temp. cortex, left	2.58	0.62	2.82	0.56	0.2
Med. inf. temp. cortex, right	2.69	0.67	2.94	0.52	0.0001
Occipital cortex, left	2.93	0.62	3.23	0.50	0.1
Occipital cortex, right	2.83	0.56	3.18	0.50	0.02
Sens. motor cortex, left	2.65	0.59	3.15	0.50	0.007
Sens. motor cortex, right	2.71	0.58	3.21	0.54	0.03
Post. cingulate, left	2.51	0.62	2.81	0.54	0.1
Post. cingulate, right	2.51	0.53	2.95	0.51	0.0005
Entorhinal cortex, left	1.25	0.43	1.50	0.45	0.09
Entorhinal cortex, right	1.08	0.24	1.42	0.35	0.001
Left hippocampus, left	0.67	0.23	0.90	0.30	0.007
Right hippocampus, right	0.63	0.18	0.85	0.31	0.006

BP_1 values for [^{18}F]altanserin binding in control subjects and patients with Tourette's syndrome in all brain regions, including age-adjusted *p* values.

response to TS pathophysiology or it could be caused by a reduction in synaptic 5-HT levels. At the present time we do not know how the in-vivo [^{18}F]altanserin binding is affected by long-term changes of 5-HT levels, but acute increases in synaptic 5-HT does not affect the binding of [^{18}F]altanserin (Pinborg et al., 2004). The reduced serotonin transporter binding in TS patients reported by Muller-Vahl et al. (2005) and Heinz et al. (1998) can be speculated to be associated with changed 5-HT levels. But whereas a primary decrease in 5-HT levels is likely to be associated with a reduction in serotonin transporter (SERT), a primary reduction in SERT is more likely to result in increased synaptic 5-HT levels. Some studies have, however, supported the notion of decreased cerebral 5-HT levels in TS patients (Anderson et al., 1992), but it is unclear whether this is causally related to the presence of tics, and further, results have been contradicted (Leckman et al., 1995). To date, there has been no evidence that SSRI treatment improves tic severity (Eapen et al., 1996). Therefore, since motor behaviour and hyper-locomotion are attenuated by 5-HT$_{2A}$ receptor

antagonism (Higgins et al., 2003; Ninan and Kulkarni, 1998) one could argue that the increased 5-HT_{2A} receptor binding is related to the presence of tics, even though a clear-cut association between 5-HT_{2A} receptors and tic severity could not be demonstrated in this study.

Moreover, we were pleased to note that one study found a significantly increased frequency of the 5-HT_{2A} receptor polymorphism T102C in patients with TS with comorbid OCD compared to healthy controls (Huang et al., 2001), but it remains to be clarified, however, if this silent mutation is of any significance for 5-HT_{2A} receptor density or affinity.

We were not able to demonstrate any association between symptom severity in terms of tics and BP_1. The small variance in tic severity (18.2 ± 3.3 for motor scores) would, however, make it difficult to establish such a correlation.

A few things need to be considered as potential confounders to our findings. Systematic differences in f_1, the non-specific tracer binding and drug effects could potentially have biased our data. However, both f_1 and the binding in the reference region (non-specific binding) did not differ significantly between the two groups. Further, it should be considered whether the increase in 5-HT_{2A} receptor binding could be caused by prior or current drug treatment since only 10 of the patients were drug free at the time of examination. Except for the SSRIs none of the drugs in use are considered to have particular affinity to the serotonergic transmitter system. Some data even consider long-term treatment with SSRIs to cause a down-regulation of the cortical 5-HT_{2A} receptor sites (Gray and Roth, 2001; Van Oekelen et al., 2003) and data from our own laboratory has previously documented that high dose SSRI treatment for more than 3 months does not change 5-HT_{2A} receptor binding (Adams et al., 2005). In addition, we were not able to demonstrate any significant differences in 5-HT_{2A} receptor binding between patients with and without medication or with and without pimozide treatment.

Our findings of increased [^{18}F]altanserin 5-HT_{2A} receptor binding in TS patients indicate a role for the serotonergic system in the manifestation of tics, either directly via the striosomal compartment in striatum or through an increased dopaminergic tone. The combination of our finding of an increase in 5-HT_{2A} receptor binding and the previous case-based observation that the 5-HT_{2A} antagonist ketanserin can ameliorate tics in patients with TS (Bonnier et al., 1999) suggests that the effect of 5-HT_{2A} antagonist treatment should be investigated in a larger study.

Acknowledgements

The authors thank Dr Mary M. Robertson for her kind help and Karin Stahr and the staff at the PET and Cyclotron Unit at Rigshospitalet, Copenhagen, and the Danish Research Centre for Magnetic Resonance at Hvidovre Hospital, Copenhagen, for their assistance. This work was supported by research grants from A. P. Møller and wife Chastine McKinney Møller Foundation, The John and Birthe Meyer Foundation, The Medical Research Fund of Copenhagen, Ludvig and Sara Elsass Foundation, Hørslev Foundation, Director Jacob Madsen & wife Olga Madsens Foundation, The Augustinus Foundation, The Beckett Foundation, Foundation for Neurological Research, The Ivan Nielsen Foundation and The Villum Kann Rasmussen Foundation.

Statement of Interest

None.

References

Adams KH, Hansen ES, Pinborg LH, Hasselbalch SG, Svarer C, Holm S, Bolwig TG, Knudsen GM (2005). Patients with obsessive-compulsive disorder have increased 5-HT2A receptor binding in the caudate nuclei. *International Journal of Neuropsychopharmacology 8*, 391–401.

Anderson GM, Pollak ES, Chatterjee D, Leckman JF, Riddle MA, Cohen DJ (1992). Postmortem analysis of subcortical monoamines and amino acids in Tourette syndrome. *Advances in Neurology 58*, 123–133.

APA (1994). *Diagnostic and Statistical Manual of Mental Disorders* (4th edn). Washington, DC. American Psychiatric Association.

Bishop C, Tessmer JL, Ullrich T, Rice KC, Walker PD (2004). Serotonin 5-HT2A receptors underlie increased motor behaviors induced in dopamine-depleted rats by intrastriatal 5-HT2A/2C agonism. *Journal of Pharmacology Experimental Therapy 310*, 687–694.

Bonnier C, Nassogne MC, Evrard P (1999). Ketanserin treatment of Tourette's syndrome in children. *American Journal of Psychiatry 156*, 1122–1123.

Braun AR, Stoetter B, Randolph C, Hsiao JK, Vladar K, Gernert J, Carson RE, Herscovitch P, Chase TN (1993). The functional neuroanatomy of Tourette's syndrome: an FDG-PET study. I. Regional changes in cerebral glucose metabolism differentiating patients and controls. *Neuropsychopharmacology 9*, 277–291.

Cheon KA, Ryu YH, Namkoong K, Kim CH, Kim JJ, Lee JD (2004). Dopamine transporter density of the basal ganglia assessed with [^{123}I]IPT SPECT in drug-naive children with Tourette's disorder. *Psychiatry Research 130*, 85–95.

Comings DE (1990). Blood serotonin and tryptophan in Tourette syndrome. *American Journal of Medical Genetics 36*, 418–430.

Comings DE, Comings BG (1987a). A controlled study of Tourette syndrome. IV. Obsessions, compulsions, and schizoid behaviours. *American Journal of Human Genetics 41*, 782–803.

Comings BG, Comings DE (1987b). A controlled study of Tourette syndrome. V. Depression and mania. *American Journal of Human Genetics 41*, 804–821.

DeGrado TR, Turkington TG, Williams JJ, Stearns CW, Hoffman JM, Coleman RE (1994). Performance characteristics of a whole-body PET scanner. *Journal of Nuclear Medicine 35*, 1398–1406.

Edgington ES (1995). *Randomization Tests* (3rd edn). New York: Marcel Dekker.

Eapen V, Trimble MR, Robertson MM (1996). The use of fluoxetine in Gilles de la Tourette syndrome and obsessive compulsive behaviours: preliminary clinical experience. *Progress in Neuropsychopharmacology and Biological Psychiatry 20*, 737–743.

Gray JA, Roth BL (2001). Paradoxical trafficking and regulation of 5-HT(2A) receptors by agonists and antagonists. *Brain 56*, 441–451.

Graybiel AM, Canales JJ (2001). The neurobiology of repetitive behaviours: clues to the neurobiology of Tourette syndrome. *Advances in Neurology 85*, 123–131.

Heinz A, Knable MB, Wolf SS, Jones DW, Gorey JG, Hyde TM, Weinberger DR (1998). Tourette's syndrome: [I-123]beta-CIT SPECT correlates of vocal tic severity. *Neurology 51*, 1069–1074.

Higgins GA, Enderlin M, Haman M, Fletcher PJ (2003). The 5-HT2A receptor antagonist M100,907 attenuates motor and 'impulsive-type' behaviours produced by NMDA receptor antagonism. *Psychopharmacology (Berlin) 170*, 309–319.

Huang Y, Liu X, Li T, Guo L, Sun X, Xiao X, Ma X, Wang Y, Collier DA (2001). Case-control association study and transmission disequilibrium test of T102C polymorphism in 5HT2A and Tourette syndrome [Abstract only]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi 18*, 11–13.

Kuroki T, Meltzer HY, Ichikawa J (2003). 5-HT2A receptor stimulation by DOI, a 5-HT2A/2C receptor agonist, potentiates amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Brain Research 972*, 216–221.

Kwak C, Vuong KD, Jankovic J (2003). Migraine headache in patients with Tourette syndrome. *Archives of Neurology 60*, 1595–1598.

Leckman JF (2002). Tourette's syndrome. *Lancet 360*, 1577–1586.

Leckman JF, Goodman WK, Anderson GM, Riddle MA, Chappell PB, McSwiggan-Hardin MT, McDougle CJ, Scahill LD, Ort SI, Pauls DL, et al. (1995). Cerebrospinal fluid biogenic amines in obsessive compulsive disorder, Tourette's syndrome, and healthy controls. *Neuropsychopharmacology 12*, 73–86.

Lewellen TK, Kohlmeyr SG, Miyaoka RS, Kaplan MS, Stearns CW, Schubert SF (1996). Investigation of the performance of the General Electric ADVANCE positron emission tomograph in 3D mode. *IEEE Transactions on Nuclear Science 43*, 2199–2206.

Lopresti B, Holt D, Mason NS, Huang Y, Ruszkiewicz J, Perevuznik J, Price J, Smith G, Davis J, Mathis C (1998). Characterization of the radiolabeled metabolites of [18F]altanserin: implications for kinetic modeling. In: Carson RE, Daube-Witherspoon ME, Herscovitch P (Eds.), *Quantitative Functional Brain Imaging with Positron Emission Tomography*, pp. 293–298. San Diego: Academic Press.

Mueller-Gaertner HW, Links JM, Prince JL, Bryan RN, McVeigh E, Leal JP, Davatzikos C, Frost JJ (1992). Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. *Journal of Cerebral Blood Flow and Metabolism 12*, 571–583.

Muller-Vahl KR, Meyer GJ, Knapp WH, Emrich HM, Gielow P, Brucke T, Berding G (2005). Serotonin transporter binding in Tourette syndrome. *Neuroscience Letters 385*, 120–125.

Ninan I, Kulkarni SK (1998). 5-HT2A receptor antagonists block MK-801-induced stereotypy and hyperlocomotion. *European Journal of Pharmacology 358*, 111–116.

Peterson BS, Skudlarski P, Anderson AW, Zhang H, Gatenby JC, Lacadie CM, Leckman JF, Gore JC (1998). A functional magnetic resonance imaging study of tic suppression in Tourette syndrome. *Archives of General Psychiatry 55*, 326–333.

Pinborg LH, Adams KH, Svarer C, Holm S, Hasselbalch SG, Haugbol S, Madsen J, Knudsen GM (2003). Quantification of 5-HT2A receptors in the human brain using [18F]altanserin-PET and the bolus/infusion approach. *Journal of Cerebral Blood Flow and Metabolism 23*, 985–996.

Pinborg LH, Adams KH, Yndgaard S, Hasselbalch SG, Holm S, Kristiansen H, Paulson OB, Knudsen GM (2004). [18F]altanserin binding to human 5HT2A receptors is unaltered after citalopram and pindolol challenge. *Journal of Cerebral Blood Flow and Metabolism 24*, 1037–1045.

Quarantelli M, Berkouk K, Prinster A, Landeau B, Svarer C, Balkay L, Alfano B, Brunetti A, Baron JC, Salvatore M (2004). Integrated software for the analysis of brain PET/SPECT studies with partial-volume-effect correction. *Journal of Nuclear Medicine 45*, 192–201.

Regeur L, Pakkenberg B, Fog R, Pakkenberg H (1986). Clinical features and long-term treatment with pimozide in 65 patients with Gilles de la Tourette's syndrome. *Journal of Neurology, Neurosurgery and Psychiatry 49*, 791–795.

Robertson MM (2000). Tourette syndrome, associated conditions and the complexities of treatment. *Brain 123*, 425–462.

Sadzot B, Lemaire C, Maquet P, Salmon E, Plenevaux A, Degueldre C, Hermanne JP, Guillaume M, Cantineau R, Comar D (1995). Serotonin 5HT2 receptor imaging in the human brain using positron emission tomography and a

new radioligand, [18F]altanserin: results in young normal controls. *Journal of Cerebral Blood Flow and Metabolism 15*, 787–797.

Saka E, Graybiel AM (2003). Pathophysiology of Tourette's syndrome: striatal pathways revisited. *Brain & Development 25* (Suppl. 1), S15–S19.

Scahill L, Leckman JF, Schultz RT, Katsovich L, Peterson BS (2003). A placebo-controlled trial of risperidone in Tourette syndrome. *Neurology 60*, 1130–1135.

Singer HS, Szymanski S, Giuliano J, Yokoi F, Dogan AS, Brasic JR, Zhou Y, Grace AA, Wong DF (2002). Elevated intrasynaptic dopamine release in Tourette's syndrome measured by PET. *American Journal of Psychiatry 159*, 1329–1336.

Smith GS, Dewey SL, Brodie JD, Logan J, Vitkun SA, Simkowitz P, Schloesser R, Alexoff DA, Hurley A, Cooper T, Volkow ND (1997). Serotonergic modulation of dopamine measured with [^{11}C]raclopride and PET in normal human subjects. *American Journal of Psychiatry 154*, 490–496.

Stamenkovic M, Schindler SD, Asenbaum S, Neumeister A, Willeit M, Willinger U, de Zwaan M, Riederer F, Aschauer HN, Kasper S (2001). No change in striatal dopamine re-uptake site density in psychotropic drug naive and in currently treated Tourette's disorder patients: a [(^{123})I]-beta-CIT SPECT-study. *European neuropsychopharmacology 11*, 69–74.

Stern E, Silbersweig DA, Chee KY, Holmes A, Robertson MM, Trimble M, Frith CD, Frackowiak RS, Dolan RJ (2000). A functional neuroanatomy of tics in Tourette syndrome. *Archives of General Psychiatry 57*, 741–748.

Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, Holm S, Paulson OB, Knudsen GM (2005). MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage 24*, 969–979.

Turjanski N, Sawle GV, Playford ED, Weeks R, Lammerstma AA, Lees AJ, Brooks DJ (1994). PET studies of the presynaptic and postsynaptic dopaminergic system in Tourette's syndrome. *Journal of Neurology, Neurosurgery, and Psychiatry 57*, 688–692.

van Dyck CH, Tan PZ, Baldwin RM, Amici LA, Garg PK, Ng CK, Soufer R, Charney DS, Innis RB (2000). PET quantification of 5-HT2A receptors in the human brain: a constant infusion paradigm with [18F]altanserin. *Journal of Nuclear Medicine 41*, 234–241.

Van Oekelen D, Luyten WH, Leysen JE (2003). 5-HT2A and 5-HT2C receptors and their atypical regulation properties. *Life Sciences 72*, 2429–2449.

Vollenweider FX, Vontobel P, Hell D, Leenders KL (1999). 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man – a PET study with [^{11}C]raclopride. *Neuropsychopharmacology 20*, 424–433.

Willendrup P, Pinborg LH, Hasselbalch SG, Adams KH, Stahr K, Knudsen GM, Svarer C (2004). Assessment of the precision in co-registration of structural MR-images and PET-images with localized binding. *International Congress Series 1265*, 275–280.

Woods RP, Cherry SR, Mazziotta JC (1992). Rapid automated algorithm for aligning and reslicing PET images. *Journal of Computer Assisted Tomography 16*, 620–633.