

From THE DEPARTMENT OF CLINICAL NEUROSCIENCE  
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# **MOLECULAR IMAGING OF THE SEROTONIN SYSTEM IN HUMAN BEHAVIOUR**

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Institutet**

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Cover images: Summation images showing regional radioactivity after intravenous injection of [ $^{11}\text{C}$ ]WAY100635 (left) and [ $^{11}\text{C}$ ]MADAM (right), transversal projection.  
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## ABSTRACT

The serotonin (5-HT) neurotransmission system has a role in essential physiological and psychological functions such as sleep, eating behaviour, sexuality, perception, emotion and cognition. Individual measures of serotonin biomarkers in the living human brain can be obtained using positron emission tomography (PET) imaging, direct measures of. The interindividual variability in these measures can then be related to human behavioural output. In combination with genotyping, this strategy provides an approach to complex chain of events linking the genetic endowment to higher brain functions. The present thesis includes a series of PET studies aimed to increase understanding of the role of serotonin in higher brain functions in man.

In study I, fifteen control subjects were examined with PET and [<sup>11</sup>C]WAY100635, a radioligand that is selective for the serotonin 5-HT<sub>1A</sub> receptor. Personality traits were assessed with the Temperament and Character Inventory (TCI) self-report questionnaire. Availability of 5-HT<sub>1A</sub> receptor was found to correlate inversely with scores for self-transcendence, a personality trait covering religious behaviour and attitudes. This finding indicates that the serotonin system may serve as a biological underpinning for spiritual experiences. Interestingly, the observed several-fold variability in 5-HT<sub>1A</sub> receptor density may partly explain why people vary greatly in spiritual zeal.

In study II, twenty-four control subjects were examined with [<sup>11</sup>C]WAY100635 PET and a battery of tests covering all major cognitive domains. There were no significant correlations between regional 5-HT<sub>1A</sub> binding and cognitive performance. The results do not provide support for involvement of the 5-HT<sub>1A</sub> receptor in cognitive functioning in man, thereby questioning the predictive validity of some currently used animal models in translational neuroscience.

In study III, seventeen volunteers were examined with [<sup>11</sup>C]MADAM PET and a battery of cognitive tests. Serotonin transporter (5-HTT) availability in cortical regions correlated significantly with performance in sustained attention. The results support a role for the serotonin system in some but not all cognitive functions in man.

In study IV, fifty-four control subjects were examined with [<sup>11</sup>C]WAY100635 PET and a battery of cognitive tests. Subjects were genotyped for the 5-HTT linked polymorphic region (5-HTTLPR), a functional polymorphism in the promoter region of the 5-HTT gene. The short (S) allele of the 5-HTTLPR has been associated with depression as well as anxiety-related personality traits. Carriers of the S allele of the 5-HTTLPR did not differ from non-carriers with respect to [<sup>11</sup>C]WAY100635 binding potential in any of the brain regions studied. S-carriers however performed significantly better in the Wisconsin Card Sorting Test. These observations suggest that functional implications of the 5-HTTLPR are not likely to be mediated by differences in 5-HT<sub>1A</sub> density and that other biomarkers must be considered for future investigations at phenotype level.

The present studies of serotonergic biomarkers in man provide new evidence for a role of the serotonin system in complex human behaviour such as cognitive functions and personality traits.



*Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.*

Marie Curie



## LIST OF PUBLICATIONS

- I. Borg J, Andréé B, Soderstrom H, Farde L. The Serotonin system and spiritual experiences. *American Journal of Psychiatry* 2003 Nov;160(11):1965-9.
- II. Borg J, Andréé B, Lundberg J, Halldin C, Farde L. Search for correlations between serotonin 5-HT<sub>1A</sub> receptor expression and cognitive functions – a strategy in translational psychopharmacology. *Psychopharmacology* 2006 Apr;185(3):389-94.
- III. Borg J, Lundberg J, Kizling I, Halldin C, Farde L. The serotonin transporter in the human brain has a putative role in attention – a PET study with [<sup>11</sup>C]MADAM. *Submitted*.
- IV. Borg J, Henningsson S, Saijo T, Inoue M, Bah J, Lundberg J, Jovanovic H, Andréé B, Halldin C, Eriksson E, Farde F. Serotonin transporter genotype is associated with cognitive performance but not regional 5-HT<sub>1A</sub> receptor binding in man. *Manuscript*.

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## LIST OF ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxy tryptamine (serotonin)
5-HTP	5-hydroxytryptophan
5-HTT	serotonin transporter
5-HTTLPR	5-HTT linked polymorphic region
8-OH-DPAT	8-Hydroxy-2-(di-n-propylamino)tetralin
AADC	aromatic amino acid decarboxylase
AC-PC	anterior-posterior commissural
BBB	blood brain barrier
BDNF	brain derived neurotrophic factor
BP	binding potential
CNS	central nervous system
CSF	cerebrospinal fluid
DA	dopamine
DASB	3-amino-4-(2-dimethylaminomethylphenylthio)benzonitrile
DAT	dopamine transporter
DRN	dorsal raphe nuclei
ECG	electrocardiogram
FWHM	full width at half maximum
GABA	gamma-aminobutyric acid
GAD	generalised anxiety disorder
keV	1 kiloelectron volt ( $1.60217646 \times 10^{-16}$ joules)
L	long (16 repeats) allele of the 5-HTTLPR
LSD	lysergic acid diethylamide
MADAM	N,N-Dimethyl-2-(2-amino-4-methylphenylthio)benzylamine
MAO	monoamine oxidase
McN 5652	(+)-6 $\beta$ -(4-methylthiophenyl)-1,2,3,5,6 $\alpha$ ,10 $\beta$ -hexahydropyrrolo-[2,1-a]-isoquinoline
MDL 100,907	(R)-(+)-4-[1-hydroxy-1-(2,3-dimethoxyphenyl)methyl]-N-2-(4-fluorophenylethyl)-piperidine
MRI	magnetic resonance imaging
MRN	median raphe nuclei
mRNA	messenger ribonucleic acid
NE	norepinephrine
NET	norepinephrine transporter
NMDA	N-methyl-D-aspartic acid
NMSP	N-methylspiperone
PET	positron emission tomography
PMDD	premenstrual dysphoric disorder
ROI	region of interest
S	short (14 repeats) allele of the 5-HTTLPR
SPECT	single photon emission tomography
SRTM	simplified reference tissue model
SSRI	selective serotonin reuptake inhibitor

TAC	time activity curve
Try OH	tryptophan hydroxylase
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WAY 100635	N-[2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl]-N-(2-pyridinyl)- cyclohexanecarboxamide trihydrochloride

# INTRODUCTION

Serotonin (5-HT) was one of the first neurotransmitters to appear during phylogenesis<sup>1</sup> and is present in almost all species from molluscs to man. In mammals, central serotonergic neurons originate from the raphe nuclei in the brain stem and project to target areas including hypothalamus, the limbic system, striatum and neocortex.

Experimental animal studies and peripheral measurements in humans have demonstrated that the serotonin system has a role in important physiological and psychological functions such as the regulation of sleep and temperature, sexuality, perception, emotion and cognition. The multiple roles suggested for serotonin in the human brain are based partly on the widespread distribution of serotonin in the nervous system as well as the presence of multiple receptor subtypes, each with a distinct expression pattern. For investigation of higher cognitive functions, animal models however have obvious limitations. In humans, peripheral measurements of 5-HT metabolites and platelet studies have been suggested as indices for central 5-HT activity. Such approaches do however suffer from validity problems since the actual relationship to serotonin activity in the brain is not known.

The development of brain imaging techniques such as positron emission tomography (PET) has paved the way for studies on the biological underpinning of human behaviour. With molecular imaging and selective radioligands, direct measurements of serotonin biomarkers in the living human brain can be obtained and related to functional human behaviour in the form of cognitive functions and personality traits. Furthermore, in combination with genotyping, the PET methodology provides a strategy to approach the complex chain of events linking the genetic endowment to higher brain functions.

The overall aim of the present thesis was to increase understanding of the role for the serotonin system in cognitive functions in man by utilising molecular (PET) imaging of serotonin biomarkers. The thesis work also extends into exploration of putative associations between genotype, biochemical phenotype and phenotype output.

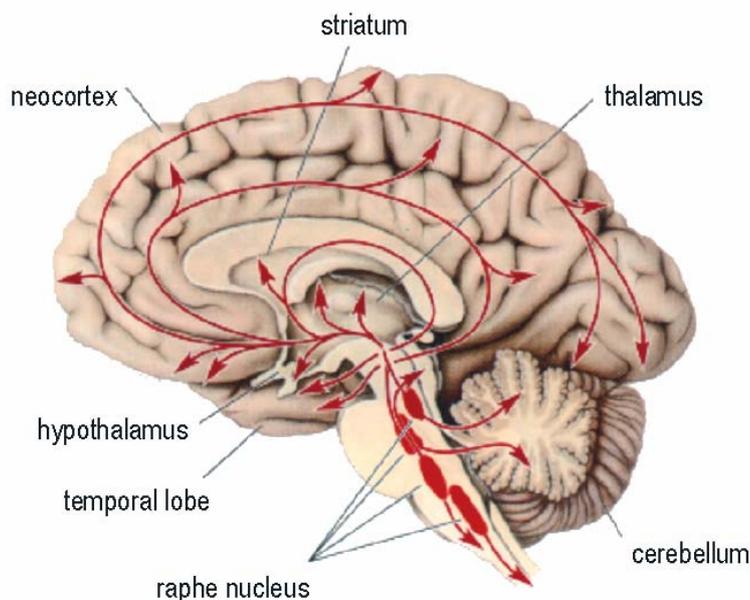
## The serotonin system

In 1868, scientists were aware of a substance in serum known to cause vasoconstriction. The identity of this factor remained unknown until the 1930's, Page, while investigating causes of hypertension, isolated a substance and named it serotonin. The name serotonin indicates its origin from blood *serum* and its effect on vascular muscle *tone*<sup>2</sup>. In Italy, Erspamer independently discovered a substance in gastric mucosa that likewise had a contractile effect on vascular and other smooth muscles<sup>3</sup>. This substance was initially called enteramine, because it was secreted by *enterochromaffin* cells of the gastrointestinal tract. In 1952, the two substances were found to be identical<sup>4</sup> and they are now exclusively referred to as serotonin, 5-hydroxytryptamine (5-HT).

The 5-HT system is one of the first neurotransmitter systems in the phylogenesis. Estimates based on analysis of homologous proteins in various species, suggest that the earliest primordial serotonin receptors appeared 700-800 million years ago, long before dopamine, muscarine and adrenergic receptor systems. Serotonin is present in blood and intestinal tract of virtually every vertebrate species, as well as in tissue of numerous invertebrates<sup>5</sup> and plants<sup>6</sup>. Studies in humans have shown that 5-HT is widely distributed in different organ systems, including the nervous system<sup>7</sup>.

### ***Serotonergic pathways in the brain***

The first anatomical description of 5-HT pathways in the mammalian central nervous system (CNS) was published in 1964<sup>8</sup>. The 5-HT cell bodies are concentrated to the raphe nuclei near the midline of the brainstem. The axonal terminals of these cell clusters innervate virtually all regions of the CNS. The 5-HT neurons in the rostral raphe nuclei, which primarily consists of the dorsal raphe nuclei (DRN) and the median raphe nuclei (MRN), project rostrally and innervate structures such as the cerebellar cortex, thalamus, striatum and the cerebral cortex<sup>9</sup>.



*FIGURE 1. Serotonergic pathways in the human brain. Mediosagittal view showing distribution of 5-HT containing cell bodies within the raphe nuclei and major ascending projections.*

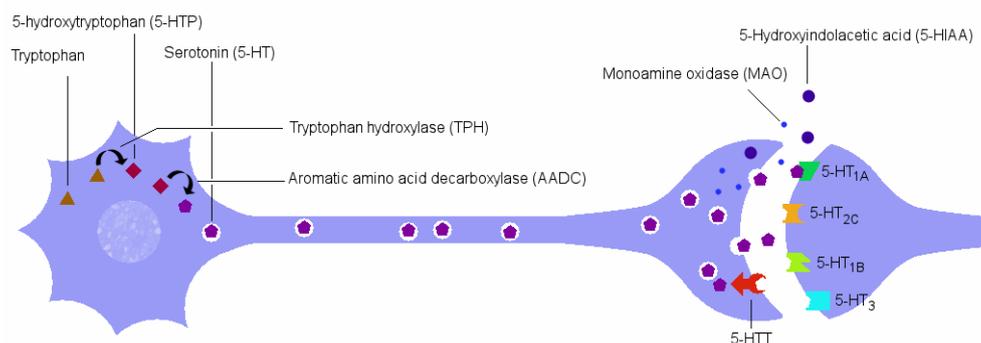
The human DRN is a well defined heterogeneous group of neurons that contains cell bodies of about 250 000 neurons<sup>10 11 12 13</sup>. Axons of these neurons project to forebrain structures throughout two major pathways. The lateral cerebral cortex is mainly innervated via a lateral pathway through the capsula interna. Medial cortical regions including the hippocampal complex, hypothalamus, basal forebrain and amygdala are all innervated via a medial pathway through the medial forebrain (Figure 1)<sup>14 15</sup>.

## **Synthesis and degradation of serotonin**

Serotonin is found in many cells that are not neurons, primarily platelets, mast cells and enterochromaffin cells. Only about 1-2% of the body content of 5-HT is located in the central nervous system, whereas 8-10% is in blood platelets and 90% is believed to exist in the mucous membranes of the gastrointestinal system. Since 5-HT cannot cross the blood brain barrier (BBB), neurons have to synthesise their own supply of 5-HT. Serotonin is produced from the amino acid precursor tryptophan. Tryptophan is first converted to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase (Try OH). 5-HTP is converted further into 5-HT by the enzyme aromatic amino acid decarboxylase (AADC), and stored in vesicles for release (Figure 2).

Serotonin is degraded by the mitochondrial enzyme monoamine oxidase (MAO) and converted into inactive metabolites. The major metabolite is 5-hydroxyindoleacetic acid (5-HIAA), which diffuses out of the neuron and to some degree enters the cerebrospinal fluid (CSF).

The concentration of 5-HT in the synaptic cleft is regulated by the serotonin transporter (5-HTT), a presynaptic membrane transport mechanism selective for 5-HT. Synaptic 5-HT is transported back by 5-HTT, into the presynaptic neuron.



*FIGURE 2. Schematic illustration of a serotonergic neuron with its cell body in the raphe nuclei (left) and axonal projection to a region with postsynaptic receptors (right). The major steps in synthesis, release and degradation of serotonin are indicated. (Note that all 5-HT receptors are not expressed in all postsynaptic neurons.)*

## **Biomarkers for serotonergic neurotransmission**

The 5-HT system is one of the most widely distributed and several proteins are known to be essential for 5-HT neurotransmission. The effects of 5-HT are mediated through binding to a variety of membrane bound receptors<sup>16,17</sup>. Fifteen 5-HT receptor subtypes, divided into seven families, have been identified<sup>18,19,20,21,22,23</sup>. Except for the 5-HT<sub>3</sub> receptor, which is a ligand gated ion-channel receptor, all known 5-HT receptors are G-protein coupled. A key protein within the serotonin system is the serotonin transporter (5-HTT). The 5-HTT is the primary mechanism

for regulation of synaptic serotonin concentration and may thus serve as a marker for global serotonergic neurotransmission activity in brain.

The present thesis focuses on two biomarkers for 5-HT neurotransmission, the 5-HT<sub>1A</sub> receptor and the 5-HTT.

### ***The 5-HT<sub>1A</sub> receptor***

The most extensively investigated 5-HT receptor is the 5-HT<sub>1A</sub> receptor. Presynaptic 5-HT<sub>1A</sub> receptors are somatodendritic autoreceptors that are highly concentrated on serotonergic cell bodies in the dorsal raphe nucleus. The presynaptic receptors constitute an important negative feed-back influence on neuronal firing, thus mediating inhibition of 5-HT release<sup>24,25</sup>. Postsynaptic 5-HT<sub>1A</sub> receptors have the highest density in the in hypothalamus, limbic and neocortical regions<sup>26,27,28</sup>. The postsynaptic receptors appear to be located on different cell types, including excitatory pyramidal and granule cells<sup>29</sup> and GABAergic inhibitory interneurons<sup>30</sup>. Due to its modulating property for the whole serotonin system and its global distribution throughout the brain, the 5-HT<sub>1A</sub> receptor may serve as a marker for a major part of serotonergic innervation in brain.

The 5-HT<sub>1A</sub> receptor has been linked to a variety of behaviours as well as to psychiatric disorder. In animal models, the behavioral effects of serotonin can be influenced or inhibited by 5-HT<sub>1A</sub> antagonists. In clinical studies, partial 5-HT<sub>1A</sub> receptor agonists such as buspirone have been shown superior to placebo for the treatment of depression, generalised anxiety disorder (GAD) and premenstrual dysphoric disorder (PMDD)<sup>31,32,33,34,35,36</sup>. Another interesting observation regarding the presynaptic 5-HT<sub>1A</sub> receptor is that pindolol, a compound that antagonises 5-HT<sub>1A</sub> mediated response in raphe nuclei<sup>37,38</sup>, may facilitate improvement of depressed patients treated with selective serotonin reuptake inhibitors (SSRI)<sup>39,40</sup>. Thus, the 5-HT<sub>1A</sub> receptor has been devoted a key role in hypotheses on pharmacological treatment of depression and anxiety<sup>41,42,43,44</sup>.

The 5-HT<sub>1A</sub> receptor has moreover also attracted interest in research on major psychiatric disorders. Postmortem studies of brains from patients with depression and schizophrenia have demonstrated an elevated 5-HT<sub>1A</sub> receptor binding in neocortex<sup>45,46,47</sup>. In PET studies of medication free patients with depression significantly decreased [<sup>11</sup>C]WAY100635 binding have been reported when compared to controls<sup>48,49</sup>.

Initial studies with PET and the selective radioligand [<sup>11</sup>C]WAY100635 have shown that the variability in density of 5-HT<sub>1A</sub> receptor in healthy volunteers is several-fold<sup>50</sup>. In the present thesis, this interindividual variability served as a starting point for the investigation of relationships between 5-HT<sub>1A</sub> receptor and functional behaviour, a strategy that has been pioneered for the dopamine system<sup>51</sup>.

### ***The 5-HTT***

The serotonin transporter (5-HTT) is a key protein within the serotonin system. The 5-HTT protein is the primary mechanism for regulation of synaptic serotonin concentration and has long been implicated in hypotheses on the pathophysiology of bipolar disorder, depression, suicide and anxiety<sup>52</sup> (for review see Arango et al 2002).

Furthermore, the 5-HTT is the primary target for selective serotonin reuptake inhibitors (SSRIs), which are established drugs for affective and anxiety disorders<sup>53</sup>. Consequently, research on 5-HTT has been largely driven by pharmacological and clinical research on antidepressant and anxiolytic drugs, whereas there is less understanding of the general role of 5-HTT in normal brain physiology and cognitive functions.

The concentration of 5-HTT is high in the raphe, putamen, hippocampal formation, cingulate cortex and frontal cortex<sup>54 55 56 57 58</sup>.

The 5-HTT has recently caught additional interest in genetic association studies. A functionally important 5-HTT promoter region linked polymorphism (5-HTTLPR) has been associated with several psychiatric disorders<sup>59</sup> (see below, page XX).

Initial PET studies of the 5-HTT in the human brain demonstrate a several-fold interindividual variability in regional protein expression<sup>60</sup>. In the present thesis, this variability served as a starting point in search for a putative association between 5-HTT protein expression in the human brain and cognitive functions.

## **Serotonin and human behaviour**

The discovery of 5-HT in the central nervous system<sup>61</sup> (CNS) in 1953 was soon followed by reports that 5-HT was a putative neurotransmitter<sup>62 63</sup>. The early antipsychotic drug reserpine was shown to reduce brain 5-HT content<sup>64</sup> and platelet 5-HT levels were altered by the antidepressants imipramine and iproniazid<sup>65</sup>. These findings led to a tremendous interest in the possible involvement of 5-HT in abnormal behavioural functioning and as site of action of psychotropic drugs. Many studies have since demonstrated the importance of 5-HT for a variety of major psychiatric disorders such as depression<sup>66,67</sup>, anxiety<sup>68,69,70,71</sup> and schizophrenia<sup>72</sup> as well as normal behaviour<sup>73,74</sup> (for review, see Jacobs & Azmitia, 1992).

### ***Serotonin hypotheses for personality***

A starting point for the 5-HT hypotheses in relation to personality disorder was the wide research initiated by an observation in 1976, where it was found that 5-HIAA in CSF was reduced in depressed patients who had attempted violent suicide<sup>75</sup>. The relationship between low levels of 5-HIAA in CSF and behavioural aspects has since then been widely investigated.

In nonhuman primates, low CSF 5-HIAA has been associated with impaired impulse control, excessive aggression and disturbances of social behaviour<sup>76</sup>. In human subjects, reduced CSF 5-HIAA has been found in subgroups of depressed patients, in patients with a family history of alcoholism, in children to alcohol abusing parents and in violent offenders with antisocial behaviour<sup>77 78 79</sup>. In these populations, low 5-HIAA has also been related to impulsive and externally aggressive behaviour<sup>80 81</sup>. The findings of low 5-HIAA in CSF in patients with depression has been reported over many years, but other studies fail to replicate this finding. This discrepancy/inconsistency may be due to the fact that 50% of CSF 5-HIAA comes from peripheral sources, which might not reflect central serotonergic functioning.

Other early findings of a relationship between serotonin and personality include the association between MAO and sensation seeking personality traits<sup>82,83</sup>. Platelets cannot synthesise 5-HT, but they share many features with 5-HT neurons, including uptake, 5-HT<sub>2A</sub> receptors and presence of MAO-B. Since the 1950's, platelets have been used for studies of antidepressants, and platelet MAO-B has been investigated as a trait marker for psychopathology, since it is encoded by the same gene as brain MAO-B<sup>84</sup>. Low MAO-B activity in platelets has been related to schizophrenia, bipolar disorder, suicidality, alcoholism and anxiety<sup>85,86,87,88,89</sup>. These findings however lack in specificity and include the possibility of environmentally induced changes in enzyme activity, why platelet studies have to be considered with some limitations.

From clinical as well as from recent genetic studies, a relationship between serotonin and anxiety has also been firmly established<sup>90,91,92</sup>. The 5-HTTLPR has been associated with anxiety related traits in several studies<sup>93,94</sup>. (Taken together, the literature supports a relationship between serotonin and anxiety and impulsive aggression.)

### ***Serotonin hypotheses for cognitive functions***

A role for the central serotonin system in cognition has been suggested from experimental animal studies<sup>95</sup>. The 5-HT<sub>1A</sub> receptor has (for several reasons) been proposed as a putative target for the development of cognitive enhancers in treatment of cognitive dysfunction in schizophrenia<sup>96</sup>, Alzheimer's disease and aging related memory decline<sup>97</sup> and traumatic brain injury<sup>98</sup>.

In Alzheimer's disease, cholinergic loss in the hippocampus and neocortex occurs in association with cognitive decline. It is hypothesised from animal experiments that 5-HT<sub>1A</sub> projections are inhibitory on the target cells for which cholinergic projections are excitatory. A suggested pharmacological approach is that 5-HT<sub>1A</sub> receptor antagonism indirectly compensate for loss of excitatory tone by blocking inhibitory tone via enhancement of glutaminergic transduction<sup>99,100</sup>. In vitro, 5-HT<sub>1A</sub> antagonists have been demonstrated to inhibit excitatory neurotransmission and release of glutamate. In animal experiments using passive avoidance tests and maze tests as models for cognitive dysfunction in Alzheimer's disease, blockade of the 5-HT<sub>1A</sub> receptor has been demonstrated to reverse the effects of cognitive deficits induced by manipulation of the cholinergic or glutamatergic system<sup>101</sup>. Moreover, experimental rodent and marmoset data have shown that antagonism of 5-HT<sub>1A</sub> receptors ameliorate cognitive impairment induced by fornix transection or the NMDA antagonist dizocilpine<sup>102</sup> (Korsic Harder et al 1996). Taken together, this line of evidence from in vitro data and in vivo animal experiments predicts a role for the 5-HT<sub>1A</sub> receptor in Alzheimer's disease.

Another line of mainly pharmacological research suggests that not only 5-HT<sub>1A</sub> antagonists but also 5-HT<sub>1A</sub> agonists may improve cognitive functioning. The agonist 8-OH-DPAT has been shown to reverse cognitive impairment induced by scopolamine<sup>103</sup>. In schizophrenia research, it has been noted that some atypical antipsychotic drugs that may enhance cognition, such as sertindole, risperidone, aripiprazole, clozapine, olanzapine, ziprasidone and quetiapine, are either 5-HT<sub>1A</sub> antagonists or partial agonists<sup>104</sup>. Improvement in verbal memory and executive functioning has been reported in patients given the 5-HT<sub>1A</sub> partial agonist Tansospirone in addition to neuroleptic treatment<sup>105</sup>. Thus, it has been suggested that agonism of the 5-HT<sub>1A</sub> receptor may enhance cognitive function<sup>106</sup>. Despite an extensive experimental

preclinical research, it however remains unclear if the 5-HT<sub>1A</sub> receptor serves as a validated target for cognitive enhancement in man.

## **Serotonergic genes**

Studying of receptor density has long been a key strategy to increase the knowledge of the etiology and pathophysiology of neuropsychiatric disorders. Interindividual variability in receptor density has been associated not only with disease and with differences in drug treatment response<sup>107</sup> but also for personality traits and cognition in normal populations. The mechanisms for regulation of serotonin 5-HT<sub>1A</sub> receptor and 5-HTT density are however poorly understood. In animal studies it has been found that 5-HT protein expression can be influenced by neuroendocrine manipulations, but genetic factors are largely unidentified.

The human 5-HT<sub>1A</sub> receptor gene is located on chromosome 5q11.2-q13<sup>108</sup>. Nine polymorphisms of the 5-HT<sub>1A</sub> gene have been described in the literature, none of which has been associated to 5-HT<sub>1A</sub> receptor density.

The human 5-HTT gene is one of the most extensively studied in psychiatry. A common 44 base pair insertion/deletion polymorphism in the promoter region of this gene, the 5-HTT linked polymorphic region (5-HTTLPR), usually appears in one of two variants, designated long (L) and short (S), respectively. Studies on human lymphoblast cell lines expressing the two different alleles have associated the S allele with restricted transcription activity<sup>109</sup>.

Synaptic levels of serotonin may regulate the density of 5-HT<sub>1A</sub> receptors, both during development by exerting an influence on the formation of serotonergic and non-serotonergic neurons, and in the adult organism by inducing adaptive changes in receptor density. In line with this, the protein regulating these levels, 5-HTT, seems to influence 5-HT<sub>1A</sub> receptor density, as suggested by pharmacological studies<sup>110,111,112</sup> and studies of knock-out mice deficient in 5-HTT. Although the studies reported so far are not consistent, regional differences in the effects on 5-HT<sub>1A</sub> receptor density and mRNA levels have been observed<sup>113,114,115</sup> in studies using 5-HTT null mutant mice. Furthermore, the knock-out mice display a gender-dependent reduction in 5-HT<sub>1A</sub> receptors particularly in the raphe nuclei.

At a behavioural level, presence of the S allele of the 5-HTTLPR gene has been associated with a variety of psychiatric disorders including anxiety<sup>116</sup>, depression<sup>117</sup>Error! Bookmark not defined. and bipolar disorder<sup>118</sup> as well as anxiety related personality traits<sup>119,120</sup>. Carriers of the S allele have also been reported to exhibit greater neuronal activity in amygdala<sup>121</sup> and to have enhanced acquisition in fear conditioning tasks<sup>122</sup>.

Despite this literature, associations between 5-HTTLPR and cognitive dimensions have however not been extensively studied across all cognitive domains.

On the basis of these observations, the question of to what extent the 5-HTTLPR polymorphism is associated with 5-HT<sub>1A</sub> receptor density in man is relevant.

## AIMS

The overall objective of the present thesis was to increase understanding of the role for the serotonin system in human behaviour by utilising molecular imaging of serotonin biomarkers in the living human brain. The specified aims were as follows:

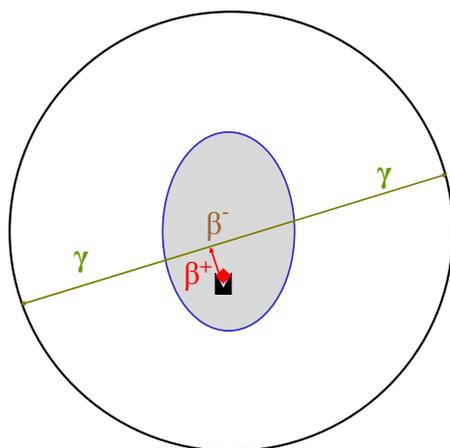
1. To investigate if interindividual variability of the serotonin 5-HT<sub>1A</sub> receptor in brain could be related to personality traits (thereby testing hypothesis of a relationship between serotonin and anxiety and aggression suggested from previous studies of peripheral serotonergic markers).
2. To investigate if predictions from current animal literature of a relationship between serotonin and cognitive functions, could be confirmed in man.
3. To explore putative associations between 5-HTTLPR genotype, serotonin biomarker availability and cognitive functions.

## MATERIALS AND METHODS

### Positron emission tomography

Positron emission tomography (PET) is a non-invasive brain imaging technique, where a tracer molecule labelled with a positron ( $\beta^+$ ) emitting radionuclide, a radioligand, is injected intravenously and transported to the brain via the blood stream. After passage of the blood brain barrier (BBB) the radioligand binds to the target molecule. At radioactive decay, an emitted  $\beta^+$  travels one to a few millimetres before annihilation with an electron ( $\beta^-$ ). The distance travelled by the  $\beta^+$  before annihilation is called  $\beta^+$  range and is dependent on the tissue in which the annihilation occurs and the  $\beta^+$  energy. The annihilation results in two 511keV  $\gamma$ -particles (photons). These travel at approximately  $180^\circ \pm 1^\circ$  and the coincidences are detected by the PET system outside the subject. The divergence from  $180^\circ$  depends on the momentum of  $\beta^+$  and  $e^-$  at the time of annihilation and together with the  $\beta^+$  range it sets the lower limit to the spatial resolution of PET systems to about 2 mm (Figure 3).[1, 2]

Coincidence events/data are corrected and reconstructed to images where the distribution of radioactivity uptake in brain tissue is computed. Regional activity is corrected for decay and plotted versus time to generate time-activity curves (TAC). Different mathematical models are then applied to the TACs to calculate biological parameters describing ligand-protein binding *in vivo*. In the present thesis, the Simplified Reference Tissue Model (SRTM) has been used for quantification of 5-HT<sub>1A</sub> receptors and 5-HTT.



**FIGURE3.** The PET principle. A radioligand (red) is bound to a receptor protein (black) within the brain (blue ellipse). After  $\beta^+$  decay annihilation with an electron ( $\beta^-$ ) occurs within a few millimetres, resulting in two  $\gamma$  particles that travel in approximately opposite directions. The coincidence is detected by sensors in the PET system (black circle). (Image courtesy of Johan Lundberg)

## Selective radioligands for the serotonin system

With PET and selective radioligands, it has over the last years become possible to examine serotonin 5-HT<sub>1A</sub><sup>123</sup> and 5-HT<sub>2A</sub><sup>124,125</sup> receptors as well as the serotonin transporter<sup>126,127</sup> (5-HTT) *in vivo* in man. These methodological developments have paved the way for more detailed investigations on the role of the central serotonin system in cognitive functioning in man.

### [<sup>11</sup>C]WAY100635

The radioligand [carbonyl-<sup>11</sup>C]WAY-100635 [N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridyl)cyclohexanecarboxamide], is an antagonist with high affinity and selectivity for 5-HT<sub>1A</sub> receptor<sup>128</sup>. The anatomical distribution in human has been extensively studied with autoradiography<sup>129</sup>, and studies on non-human primates have shown highly reduced affinity for 5-HT<sub>1A</sub> receptor after pretreatment with non marked WAY-100635 use of the reference substance 8-OH-DPAT, buspirone and pindolol<sup>130</sup>. Subsequently, it was demonstrated that WAY-100635 is also suitable for humans<sup>131</sup>.

In studies I, II and IV, [Carbonyl-<sup>11</sup>C]-WAY-100635 was prepared by <sup>11</sup>C-acylation of WAY100634 with carbonyl-<sup>11</sup>C-cyclohexanecarbonyl chloride as described previously<sup>132</sup>. A sterile phosphate buffer (pH 7.4) solution of 175-313 MBq of [<sup>11</sup>C]WAY100635 was injected intravenously at start of the PET measurement. The specific radioactivity at the time of injection was 7.4-49.5 GBq/mmol.

### [<sup>11</sup>C]MADAM

The radioligand [carbonyl-<sup>11</sup>C]MADAM [N,N-Dimethyl-2-(2-amino-4-methylphenylthio) benzyl amine] is a selective serotonin transporter inhibitor. In autoradiographic studies on rat and post-mortem human brain slices [<sup>3</sup>H]MADAM

bound in regions known to have high 5-HTT density and the binding could be inhibited by reference ligands<sup>133</sup>. It has recently been demonstrated that [<sup>11</sup>C]MADAM is suitable for *in vivo* studies in humans.

In study III, [<sup>11</sup>C]MADAM was prepared by methylation of the corresponding des-methyl precursor using [<sup>11</sup>C]methyl triflate<sup>134</sup>. A sterile phosphate buffer (pH 7.4) solution of 282-318 MBq of [<sup>11</sup>C]MADAM was injected intravenously at start of the PET measurement. The specific radioactivity at time of injection was between 5.4-78 GBq/mmol.

## Subjects

All studies were approved by the Ethics and Radiation safety committees of the Karolinska Hospital and were conducted in full compliance with the Declaration of Madrid.

Altogether 69 control subjects (53 males, 16 females), age range 20-55 years, participated in PET examination with [<sup>11</sup>C]WAY100635 or [<sup>11</sup>C]MADAM, cognitive assessment, personality assessment and genotyping after giving written informed consent. Subjects were healthy according to history, physical examination, routine blood and urine analyses and magnetic resonance imaging (MRI) of the brain. Exclusion criteria were somatic disorder, pregnancy, major psychiatric disorder according to DSM-IV, previous intake of psychotropic drugs or history of substance addiction.

## MRI and PET protocols

All subjects were examined with the MRI system Signa Advantage® 1.5 Tesla (General Electric). A standard spin-echo sequence with a 256 x 256 matrix was used with a repetition time (TR) of 4 seconds. Proton density weighted images (17 msec) and T2 weighted images (85 msec) were obtained to achieve one set of images with high spatial resolution and another with high sensitivity for pathology. Both in the MRI and the PET measurements an individual head fixation system was used to allow for the same head positioning in the two imaging modalities<sup>135</sup>.

The PET system used was Siemens ECAT Exact HR 47, run in three-dimensional mode with Dual Energy Windows scatter correction. The system covers an axial distance of 15 cm. The in-plane resolution of the reconstructed images is 3.8 mm full width at half maximum (FWHM) and the axial resolution 4.0 FWHM<sup>136</sup>. Radioactivity in brain was measured in a series of 16 consecutive frames for 69 minutes. Radioactivity in brain was measured in a series of 20 consecutive frames for 93 minutes. The frame sequence consisted of three one-minute frames followed by four three-minute frames and 13 six-minute frames. The reconstructed volume was displayed as 47 horizontal brain sections with a center to center distance of 3.125 mm.

## PET data analysis

Anatomical designation of regions of interest (ROIs) and calculations of the binding potential<sup>137</sup> (BP) were made blind to results in behavioural measurements. The ROIs were manually delineated using software developed for the Human Brain Atlas<sup>138</sup>. All ROIs but the raphe were delineated in three to five consecutive sections on the MRI images and transferred to the corresponding PET images. The raphe is not visible on MRI images and was therefore delineated as a standardised circular ROI of 6 mm in three to five consecutive sections directly on the PET images<sup>139</sup>. Data were pooled so that the average radioactivity concentration for the whole volume of interest was obtained.

To obtain time-activity curves, regional radioactivity was calculated for each frame, corrected for decay and plotted versus time. In study I, BP values for [<sup>11</sup>C]WAY100635 binding to 5-HT<sub>1A</sub> receptors were calculated for the dorsal raphe nuclei, the hippocampus and the neocortex. In study II and IV, BP values for [<sup>11</sup>C]WAY100635 binding to 5-HT<sub>1A</sub> receptors were calculated for the dorsal raphe nuclei, the hippocampus, the amygdale, the insula, the anterior cingulate, the temporal cortex and the frontal cortex. In study III, BP values for [<sup>11</sup>C]MADAM binding to 5-HTT were calculated for the dorsal raphe nuclei, the hippocampus, the insula, the anterior cingulate, the temporal cortex and the frontal cortex. In addition, the cerebellum, having negligible 5-HT<sub>1A</sub> receptor and 5-HTT expression<sup>140,141</sup>, served as a reference region in all studies. The Simplified Reference Tissue Model (SRTM)<sup>142</sup> was used to obtain BP values in all studies.

During the course of the present thesis, observations of subjects with 5-HT<sub>1A</sub> binding in the cerebellum have been reported<sup>143,144</sup>. Therefore, visual inspection of all individual PET images was performed with particular emphasis on homogeneity in cerebellum. In the literature, most of the cases with 5-HT<sub>1A</sub> binding in the cerebellum have conspicuous uptake in the vermis. Thus, although the vermis was not included in the cerebellum ROI delineation, it was visually inspected as a marker for putative cerebellum binding. In study IV, one subject with 5-HT<sub>1A</sub> binding in the cerebellum could be identified, and was accordingly excluded from the study. No support for 5-HT<sub>1A</sub> binding in cerebellum was found in any other subject.

For studies III and IV, a coregistration procedure was used. Spatial normalisation of the MR-images and coregistration of MR- and PET images was performed with the coregistration procedure in Statistical Parametric Mapping 2.0 for Windows. For each subject, the MR-images were spatially normalised to position the anterior-posterior commissural (AC-PC) line in the horizontal plane, and the interhemispheric plane orthogonal to the AC-PC plane. The reoriented MR-images were then resampled in the transaxial plane to 1 mm<sup>3</sup> voxels, and cropped to a 256x256x141 matrix. For coregistration of the PET-images to the MR-images, a 2x2x3-resolution dummy was generated. The summated PET images including individual time frames, were resampled to a 2 mm<sup>3</sup> voxel size and coregistered to the dummy.

## **Cognition**

The initial test battery, used in study II, was a clinical battery where test procedures were selected to assess cognitive domains that have been reported to be impaired in neuropsychiatric disorders where the central serotonin system has been hypothesised to play a role. Preliminary analysis of the first ten subjects however revealed ceiling effects and lack of sensitivity in the range of normal performance. Thus, in order to increase specificity and sensitivity, we extended the test battery and included ten additional tests based also on observations from the experimental animal literature. The extended battery was used for the last 14 subject in study II and for all subjects in study III.

### **Initial cognitive test procedures**

#### ***Vocabulary***

The Swedish version<sup>145</sup> of the subtest Vocabulary from the Wechsler Adult Intelligence Scale-Revised<sup>146</sup> (WAIS-R) is a global assessment of crystallised intelligence, measuring the extent of recall vocabulary and the effectiveness of speaking vocabulary. The task is to explain the meaning of a number of words presented to the subjects both written at a sheet of paper and by the examiner orally. The list consists of 40 words, arranged in order of difficulty.

#### ***Claeson-Dahl Learning and Retention Test***

A standardised Swedish verbal list learning test for assessment of auditory verbal memory, effectiveness of verbal learning, retention and retrieval<sup>147,148</sup>. Ten common Swedish two-syllable words are read to subjects at a rate of one word per second. After a delay of 15 seconds, subjects are instructed to reproduce as many words as possible. This procedure is repeated ten times, or if that occurs in less than ten trials, until subjects have reproduced all ten words twice. Twenty minutes after the learning period, subjects are asked to recall the words from memory. A recognition task where subjects choose one word from an orally presented list of three words is also given. Order memory is assessed by administration of a written list where the ten words appear in randomised order and subjects are asked to recall in which order the words were read by the examiner during the learning period.

#### ***Continuous Performance Test***

This computerised test provides a measure of vigilance and mental speed<sup>149</sup>. Four-digit numbers are flashed on the screen for 50 ms at an interval of 1 second. The distance between the subject's eyes and the screen is 50 cm. Subjects are instructed to indicate when the same number is presented twice. This is done by clicking a mouse button with the index finger of the dominant hand.

#### ***Spatial Working Memory Test***

A computerised test for examination of spatial working memory, spatial orientation and visual discrimination<sup>150</sup>. Subjects are asked to look at a small cross in the lower part of the screen. A black circle flashes briefly on the screen, and a three-digit number appears at the fixation point where the cross was. Subjects are told to count backwards

aloud, beginning with the number given. After a delay of either 5 or 15 seconds, 8 white circles appears on the screen, and subject's task is to point at the circle located at the same place as the black circle presented earlier.

### ***Rey-Osterrieth's Complex Figure***

The Complex Figure is a construction task involving copying, immediate recall and delayed recall<sup>151152</sup>. A figure consisting of basic geometrical shapes is administered, and subjects are instructed to copy the figure as correctly as possible. No time limit is given. Three minutes later subjects are asked to reproduce the figure from memory. Twenty minutes after the copying (modification from the original procedure, where the delay is 30 minutes), there is a second recall trial and subjects are asked to draw the figure from memory again.

### ***Controlled Oral Assessment***

An assessment of verbal fluency<sup>153154</sup>. In Letter fluence, the task is to say as many different words as possible, beginning with the letters F, A and S. There is a one-minute trial for each letter, and no proper nouns, numbers or same word with different suffix are allowed.

In Category fluency subjects are instructed to say as many words as possible belonging to the same category<sup>155</sup>. The categories are animals, fruits and vegetables, and one minute is allowed for each category.

### ***Wisconsin Card Sorting Test***

Computerised version of Wisconsin Card Sorting Test<sup>156157</sup>, a test of concept formation, attentional set-shifting and reasoning. On the cards are different pictures, varied by combining the three dimensions colour, symbol and number. Four reference cards are presented at the top of the screen, and new cards are presented below. Subjects are asked to sort the new cards according to the reference cards. No other instruction is given. The response "correct" or "incorrect" is given on the screen for each card sorted. The principle for sorting changes when ten correct responses have been made. A maximum of 128 cards are administered.

## **Additional test procedures**

### ***Block Design***

The Swedish version<sup>158</sup> of the subtest Block Design from WAIS-R<sup>159</sup> was used to obtain a global measurement of fluid intelligence. Subjects use blocks to construct patterns according to a pattern.

### ***Verbal Working Memory Test***

The Daneman-Carpenter reading span task<sup>160161</sup> is a dual-task procedure measuring working memory capacity. Subjects read a number of sentences (2-6) and are instructed to judge whether the sentence is correct or not. Simultaneously, subjects must memorise the last word of each sentence. At the end of each sequence, subjects repeat the last word of each sentence.

### ***Spatial Working Memory Test***

A computerised dual-task procedure for assessment of non-verbal working memory capacity<sup>162</sup>. A sequence of letters (1-8) is presented, and subjects are instructed to judge if the letter is reversed or not. Apart from being reversed or regular, letters are also rotated, and at the end of each sequence subjects indicate the position of the upper part of each letter by pointing at a paper template.

### **Cambridge Neuropsychological Test Automated Battery**

From the Cambridge Neuropsychological Test Automated Battery<sup>163</sup> (Cantab), the following test procedures were included:

#### ***Reaction Time***

The task is divided into five stages, including a single choice and a multiple choice reaction time task. In each stage, the subject is instructed to press a pad as soon as a stimulus appears. In two of the stages subjects respond both by pressing the pad and touching the screen, which allows for dividing the outcome measures into reaction time and movement time.

#### ***Rapid Visual Information Processing***

A test of visual sustained attention. A white box appears in the centre of the computer screen, inside which digits, from 2 to 9, appear in a pseudo-random order, at the rate of 100 digits per minute. Subjects are requested to detect target sequences of digits and to register responses using the press pad.

#### ***Spatial Span***

An assessment of non-verbal memory span. Nine white squares are shown, some of which briefly change colour in a variable sequence. The subject is instructed to touch the squares which changed colour in the same order that they were displayed.

#### ***Pattern Recognition Memory***

This is a test of visual pattern recognition memory in a two-choice forced discrimination paradigm. Subjects are presented with a series of 12 visual patterns, one at a time. In the recognition phase, subjects choose between a pattern they have already seen and a novel pattern. The procedure is then repeated, with 12 new patterns.

#### ***Delayed Matching to Sample***

A matching-to-sample task assessing non-verbal recognition. A complex visual pattern is displayed and after a brief or a longer delay, four similar patterns. The subject indicates which of the four patterns exactly matches the sample.

#### ***Spatial recognition***

This is a test of spatial recognition memory in a two-choice forced discrimination paradigm. A white square appears in sequence at five different locations on the screen. In the recognition phase, a series of five pairs of squares appears. In each pair, one square is in a place previously seen and the other square is in a location not seen in the presentation phase. Subjects are instructed to touch the square that is displayed in the same place as in the presentation phase.

### ***Intra-Extra Dimensional shift***

A test of attentional set-shifting and rule acquisition. Two dimensions are used in the test, coloured shapes and white lines. Simple stimuli are made up of one of these dimensions, whereas compound stimuli are made up of both. Initially, two simple coloured shapes are displayed and subjects are instructed to touch the one that is correct. After each selection, the response “correct” or “incorrect” is given on the screen. The first shifts are intra-dimensional (coloured shapes remain the only relevant dimension), then later extra-dimensional (white lines become the only relevant dimension). After six consecutive correct responses, the stimuli and/or rules are changed. A maximum of 50 trials are administered.

### ***Stockings of Cambridge***

A spatial planning test which gives a measure of executive function. The test is designed to be a computerised analogue to Tower of London. Two sets of coloured balls are displayed. Subjects are instructed to use the balls in the lower display to copy the pattern shown in the upper display. The balls are moved one at a time by touching the required ball, then touching the position to which it should be moved.

## **Personality test procedures**

### ***Temperament and Character Inventory***

The Swedish translation<sup>164</sup> of the Temperament and Character Inventory<sup>165 166</sup> (TCI) self-report questionnaire was used to assess personality traits. The TCI consists of 238 items subjects can respond either “true” or “false” to. TCI covers the four temperament dimensions of novelty seeking, harm avoidance, reward dependence, and persistence and the three character dimensions of self-directedness, cooperativeness, and self-transcendence. TCI has been developed according to a suggestion that the four temperament dimensions reflect inherited behavior, whereas the three character dimensions are thought to be influenced by environment.

## **DNA analysis**

Amplification was made by means of polymerase chain reaction (PCR) on a Perkin Elmer 9700 thermal cycler. The primer sequences used for the 5-HTTLPR, reported by Gelernter et al<sup>167</sup>, were 5'-ATGCCAGCACCTAACCCTAATGT-3' (forward primer) and 5'-GGACCGCAAGGTGGGCGGGA-3' (reverse primer), resulting in a 419-bp-long PCR product for the 16-repeat-allele (long, L), and a 375-bp-long PCR product for the 14-repeat-allele (short, S). The 15µl reaction mixture contained 50ng genomic DNA, 1.5mM MgCl<sub>2</sub>, 0.3µM of each primer, 300µM dNTPs, and 1 unit of HotstarTaq polymerase from Qiagen. The temperature profile consisted of an initial denaturation at 95°C for 15 min, followed by 45 cycles of 30s at 95°C, 30s at 66°C, and 60s at 72°C, followed by final incubation for 7 min at 72°C. PCR-products were separated on a 3% agarose gel supplemented with ethidium bromide, and visualized by ultraviolet transillumination.

## RESULTS

### Study I: The Serotonin System and Spiritual Experiences

The purpose of this positron emission tomography (PET) study was to search for relationships between serotonin 5-HT<sub>1A</sub> receptor density and personality traits.

Fifteen normal male subjects, ages 20–45 years, were examined with PET and the radioligand [<sup>11</sup>C]WAY100635. Personality traits were assessed with the Swedish version of the Temperament and Character Inventory self-report questionnaire. Binding potential, an index for the density of available 5-HT<sub>1A</sub> receptors, was calculated for the dorsal raphe nuclei, the hippocampal formation, and the neocortex. For each region, correlation coefficients between 5-HT<sub>1A</sub> receptor binding potential and Temperament and Character Inventory personality dimensions were calculated and analyzed in two-tailed tests for significance.

5-HT<sub>1A</sub> binding potential correlated inversely with scores for self-transcendence, a personality trait covering religious behavior and attitudes. No correlations were found for any of the other six Temperament and Character Inventory dimensions. The self-transcendence dimension consists of three distinct subscales, and further analysis showed that the subscale for spiritual acceptance correlated significantly with binding potential but not with the other two subscales.

This finding in normal male subjects indicated that the serotonin system may serve as a biological basis for spiritual experiences.

### Study II: Search for correlations between serotonin 5-HT<sub>1A</sub> receptor expression and cognitive functions – a strategy in translational psychopharmacology

The purpose of this positron emission tomography (PET) study was to search for relationships between interindividual variability in serotonin 5-HT<sub>1A</sub> receptor binding potential (BP) and cognitive functioning.

Twenty-four male control subjects, age 20-55 years, were examined with [<sup>11</sup>C]WAY100635 PET and a battery of cognitive tests. 5-HT<sub>1A</sub> receptor binding potential were calculated for the raphe nuclei, the hippocampus and the neocortex. Correlation coefficients between BP and cognitive performance were obtained for each region.

There was a several-fold variability in 5-HT<sub>1A</sub> BP between individuals. We found no significant correlation between regional [<sup>11</sup>C]WAY100635 binding and cognitive performance.

The results do not provide support for involvement of the 5-HT<sub>1A</sub> receptor in cognitive functioning in man and question the predictive validity of some currently used animal models in translational neuroscience.

### **Study III: The serotonin transporter in the human brain has a putative role in attention – a PET study with [<sup>11</sup>C]MADAM**

The serotonin transporter (5-HTT) is the primary mechanism for regulation of synaptic serotonin concentration and may thus provide a marker for global serotonergic neurotransmission activity in the brain. The aim of the present molecular brain imaging study was to explore if the suggested role for the serotonin system in cognitive functions could be confirmed in man.

Seventeen male volunteers, age 21-55 years, were examined with PET and the radioligand [<sup>11</sup>C]MADAM. The binding potential (BP) of [<sup>11</sup>C]MADAM to available 5-HTT was calculated for the dorsal raphe nuclei, the hippocampus, the anterior cingulate, the insula, the temporal cortex and the frontal cortex. Cognitive functions were assessed with a battery of manual and computerised tests procedures.

BP of [<sup>11</sup>C]MADAM in all projection areas but not the raphe, correlated significantly with scores in Rapid Visual Information Processing (RVIP), a procedure measuring sustained attention. For the temporal cortex, the significance remained also after Bonferroni correction for multiple comparisons. When controlling for age, level of education and global IQ with a partial correlation, we also found significant correlations between performance in Spatial Working Memory Test and BP of [<sup>11</sup>C]MADAM in raphe, anterior cingulate and hippocampus.

In accordance with animal data, the present results support a role for the serotonin system in the biological underpinning of cognitive functions in man. The observed relationship between 5-HTT expression levels and performance in an attention task may also require particular interest in future studies on the pathophysiology of major psychiatric disorder.

### **Study IV: Serotonin transporter genotype is associated with cognitive performance but not regional 5-HT<sub>1A</sub> receptor binding in man**

The human serotonin transporter (5-HTT) gene is one of the most extensively studied in psychiatry. A functional polymorphism in the promoter region of the 5-HTT gene (5-HTTLPR) has been associated with several psychiatric disorders as well as anxiety related personality traits. In search for a mechanistic understanding of the functional implications of the 5-HTTLPR, the influence of this polymorphism on regional 5-HT<sub>1A</sub> receptor expression has been examined in two PET studies in man. The reported effect goes in different directions, however.

In the present study, 54 control subjects, age 20-55 years, were genotyped and examined with [<sup>11</sup>C]WAY100635 PET and a battery of cognitive tests. The regional binding potential (BP) of [<sup>11</sup>C]WAY100635 to 5-HT<sub>1A</sub> receptor was calculated with the Simplified Reference Tissue model for the dorsal raphe nuclei, the hippocampus, the anterior cingulate, the insula, the temporal cortex and the frontal cortex.

Whereas carriers of the short (S) allele of the 5-HTTLPR did not differ from non-carriers with respect to [<sup>11</sup>C]WAY100635 binding potential in any of the brain regions studied, they performed significantly better on the Wisconsin Card Sorting Test. The results suggest that 5-HTTLPR exerts a significant influence on brain serotonergic

neurons involved in certain aspects on cognition, but that this influence is reflected neither by 5-HT<sub>1A</sub> receptor density at postsynaptic neurons, nor by 5-HT<sub>1A</sub> receptor density at serotonergic cell bodies in the raphe nuclei.

## **SIGNIFICANCE OF THE STUDIES**

Neuropsychiatric disorders such as schizophrenia, bipolar disorder and autism are defined from operational clinical criteria based on observable symptoms. A consequence of this is considerable phenotype heterogeneity that likely constitutes a major limitation for progress in understanding of the pathophysiology and pathogenesis of these disorders. A strategy to shorten the complex route between genotype and heterogeneous phenotype is to address intermediate phenotypes, *endophenotypes*. Endophenotypes are defined as elementary component parts that may provide a more direct link to the biological and genetic underpinning than the clinical syndrome itself<sup>168</sup>.

Utilisation of molecular imaging in combination with genetic analysis and measurements of functional behaviour, can aid identification of endophenotypes and genes of importance for neuropsychiatric disorder. The present thesis represents a step towards identification and evaluation of serotonergic biomarkers and cognitive functions that can serve as feasible candidate endophenotypes. Identification of endophenotypes can not only increase understanding of the pathophysiology of these disorders, but may also indicate future treatment types and predict treatment outcome.

Cognitive impairment is a feature in many common neuropsychiatric disorders. In clinical neuroscience, translational research on higher brain functions has however been hampered for several reasons such as lack of valid animal models and direct tissue accessibility. A particular aim of molecular imaging is to identify biomarkers in relation to higher brain functions or clinical symptoms for which there are no generally accepted animal models. The present thesis, attempting to confirm observations from animal models in man, represents a translational effort that may facilitate development of validated animal models of use for advancement of pharmacological treatment of cognitive impairment.

## **FUTURE PERSPECTIVES**

With fifteen receptor subtypes, each with its own distinct expression pattern, the central serotonin system is one of the most complex neurotransmission systems in the human brain. In addition to this, there are reports of splice variants, RNA edited isoforms and naturally occurring polymorphic variants which adds another dimension of complexity<sup>169,170</sup>. This complexity illustrates that 5-HT exert its multiple effects by many actions.

The present thesis, which has investigated two serotonin biomarkers in the human brain, constitutes a beginning in exploration of the role of the serotonin system in human behaviour. Although the function of many serotonin receptor subtypes remains to be investigated, some subtypes have been implicated in cognitive functions from

animal models. Future radioligand development will allow for further investigation of the functional role of other serotonergic biomarkers in man.

The genetic mechanisms for regulation of serotonin receptors are poorly understood. The present thesis investigated only one genetic polymorphisms of the 5-HTT gene, the 5-HTTLPR, with negative results. Future investigations of other serotonin related candidate genes should include also the 5-HT<sub>1A</sub> gene, the tryptophan hydroxylase gene and sex hormone genes.

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