Sex differences in the serotonin-1A receptor distribution of the human brain measured by positron emission tomography

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Marii i Rajmundowi Wierzchowskim,
których ciepło, miłość i mądrość
towarzyszyły mi przez całe życie.

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Summary

Background: It has been long known that women are twice as likely to suffer from affective illnesses as depression or anxiety than are men, while the reason for this sex difference is still unclear. Affective disorders have been frequently asssociated with alterations of the serotonergic system and in particular with alterations of the serotonin-1A ($5\text{-}HT_{1A}$) receptor. It has been suggested that sexual dimorphisms of the serotonin neurotransmission may contribute to the pathogenesis of depression and anxiety disorders.

Aim: The aim of the study was to examine sexual dimorphisms of the $5\text{-}HT_{1A}$ receptor binding potential (BP_{ND}) using positron emission tomography (PET) and the radioligand [*carbonyl*-^{11}C]WAY-100635.

Methods: Sixteen healthy male (age 26.2 ± 4.2 years, mean ± SD) and 16 healthy female (24.1 ± 2.6 years, mean ± SD) subjects were measured using a GE Advance PET camera (total acquisition time of 90 minutes). All females were measured within the follicular phase. Acquisition started simultaneously with the injection of the radioligand [*carbonyl*-^{11}C]WAY-100635 with a mean injected dose of 5.65 ± 0.8 MBq/kg body weight. The $5\text{-}HT_{1A}$ receptor BP_{ND} was quantified in regions of interest (ROIs) using a manual and an automated delineation approach. For the manual method, nine ROIs (anterior and posterior cingulate cortex, amygdala, hippocampus, insula, orbitofrontal cortex, retrosplenial cortex, hypothalamus and dorsal raphe nuclei) were drawn manually on co-registered T1-weighted MR images. For the automated method, a PET template for 45 ROIs was created based on Automated Anatomical Labelling (AAL). The $5\text{-}HT_{1A}$ receptor BP_{ND} in ROIs was calculated using the Simplified Reference Tissue Model. To evaluate the mean effect of sex on the $5\text{-}HT_{1A}$ receptor BP_{ND}, two-way analyses of variance (ANOVAs) were conducted using the BP_{ND} values as dependent variables, sex as between-subject factor, region as within-subject factor, subject as random factor and the interaction term sex by region. Furthermore, *t*-tests were conducted in each ROI to assess sex effects on the regional $5\text{-}HT_{1A}$ receptor distribution.

Results: The two-way ANOVAs revealed no significant sex differences in the $5\text{-}HT_{1A}$ receptor BP_{ND}, regardless of the method used (manual method: $F_{1,30}$= 1.3, p=.278; automated method: $F_{1,30}$= 0.8, p=.378). There was no significant interaction between sex and region, and independent samples *t*-tests revealed no significant sex differences in any region of interest (p>.05). The only exception was a trend towards a lower BP_{ND} in women in the hypothalamus (p=.012) that, however, did not withstand the Bonferroni correction for multiple comparisons.

Conclusion: In this study conducted in 32 healthy men and women, no sex differences in $5\text{-}HT_{1A}$ receptor binding were found both applying the manual delineation method and an automated delineation approach based on AAL. The findings indicate a similar $5\text{-}HT_{1A}$ receptor distribution in healthy men and women. Therefore, the higher prevalence of affective disorders in women can not be explained by a sexually dimorphic $5\text{-}HT_{1A}$ receptor binding.

Zusammenfassung

Hintergrund: Es ist schon lange bekannt, dass Frauen doppelt so häufig an affektiven Störungen wie Depression oder Angsterkrankungen leiden als Männer, die Ursache dafür ist jedoch immer noch ungeklärt. Da affektive Erkrankungen häufig mit Veränderungen im serotonergen System, und insbesondere mit Veränderungen im Serotonin-1A ($5\text{-}HT_{1A}$) Rezeptor assoziiert wurden, wird heute vermutet, dass Geschlechtsdimorphismen der serotonergen Neurotransmission an der Entstehung von Depression und Angst beteiligt sein könnten.

Ziel: In der vorliegenden Arbeit haben wir mit Hilfe der Positronen Emissions Tomographie (PET) und dem Radioliganden [*carbonyl*-^{11}C]WAY-100635 untersucht, ob sich das $5\text{-}HT_{1A}$ Bindungspotenzial in 32 gesunden Frauen und Männern unterscheidet.

Methodik: Sechzehn gesunde Männer im Alter von 26.2 ± 4.2 Jahren und sechzehn gesunde Frauen im Alter von 24.1 ± 2.6 Jahren nahmen an der Studie teil. Die Messungen erfolgten mit einem GE Advance PET Scanner (Gesamtmessdauer 90 Minuten). Alle Frauen wurden in der follikulären Phase des Zyklus untersucht. Die dynamischen Messungen starteten gleichzeitig mit der Injektion des Radioliganden [*carbonyl*-^{11}C]WAY-100635 bei einer durchschnittlichen Dosis von 5.65 ± 0.8 MBq/kg Körpergewicht. Zielregionen zur Ermittlung des $5\text{-}HT_{1A}$ Bindungsppotenzial wurden mit Hilfe einer manuellen und einer automatisierten Methode definiert. Für die manuelle Methode wurden 9 Zielregionen (das vordere und hintere Zingulum, die Amygdala, der Hippokampus, die Insula, der orbitofrontale Kortex, der retrospleniale Kortex, der Hypothalamus und die dorsalen Raphekerne) auf co-registrierten T1-gewichteten Magnetresonanzbildern eingezeichnet. Für die automatisierte Auswertung wurde auf Basis von Automated Anatomical Labelling (AAL) eine PET Vorlage für 45 Zielregionen erstellt. Das Rezeptor-Bindungspotenzial wurde mit Hilfe des "Simplified Reference Tissue Models" ermittelt. Um den Einfluss von Geschlecht auf das $5\text{-}HT_{1A}$ Bindungspotenzial statistisch zu analysieren, wurden univariate Varianzanalysen (ANOVA) mit dem Bindungspotenzial als abhängige Variable, Geschlecht und Region als feste Faktoren, Proband als Zufallsfaktor und mit der Interaktionsvariable Geschlecht x Region durchgeführt. T-Tests für unabhängige Stichproben wurden zudem in jeder Region durchgeführt, um eine eventuelle geschlechtsspezifische Rezeptorverteilung auszuschließen.

Ergebnisse: Die univariate Varianzanalyse zeigte keinerlei signifikante Geschlechtsunterschiede im $5\text{-}HT_{1A}$ Bindungspotenzial weder mit der manuellen ($F_{1,30}$= 1.3, p=.278) noch mit der automatisierten ($F_{1,30}$= 0.8, p=.378) Methode. Es gab auch keinen signifikanten Zusammenhang zwischen Geschlecht und Region und die T-Tests für unabhängige Stichproben zeigten keinerlei Geschlechtsunterschiede in den einzelnen Region (p>.05. Eine Ausnahme war der Hypothalamus, der einen Trend Richtung niedrigerem Bindungspotenzial in Frauen zeigte (p=.012), welcher aber nicht der Bonferronikorrektur für multiple Vergleiche widerstand.

Konklusion: In der vorliegenden Studie in 32 gesunden Probandinnen und Probanden wurden keine Geschlechtsunterschiede im $5\text{-}HT_{1A}$ Bindungspotenzial beobachtet, weder mit der manuellen Methode noch mit der automatisierten Methode basiserend auf AAL. Das $5\text{-}HT_{1A}$ Rezeptor Bindungspotenzial war in Frauen und Männerm in gleicher Weise verteilt. Die höhere Prävalenz affektiver Erkrankungen in Frauen kann daher nicht durch Geschlechtsdimorphismen im $5\text{-}HT_{1A}$ Rezeptor erklärt werden.

Contents

1. INTRODUCTION

Twice as many women compared to men suffer from major depression and anxiety disorders which affect nearly one fifth of the Western population and cause an immense personal, social and economic burden (see e.g. Alonso et al. 2004). The striking sex difference in prevalence rates appears to be independent of country and culture and can not be entirely explained by psychosocial factors, social support or coping style (Gater et al. 1998). Rather, it has been demonstrated that women and men differ significantly in brain structure and function (for reviews, see Cahill 2006 or Cosgrove et al. 2007) suggesting a higher biological susceptibility to depression in females.

Interestingly, a growing body of evidence suggests that the serotonergic system, which is known to be altered in affective disorders, may be sexually dimorphic. In the brain of female rodents, a higher tryptophan content and utilization rate (Carlsson et al. 1985), a higher serotonin synthesis and serotonin turnover (Haleem et al. 1990) and overall higher serotonin levels (Dickinson and Curzon 1986) were demonstrated. Several human studies reported a greater responsiveness to serotonergic challenges in female participants (McBride et al. 1990). Acute tryptophan depletion, used as an experimental model for depression, was shown to affect females to a significantly larger extent than males (Sambeth et al. 2007). Also, *in vivo* assessment of serotonergic structure and function with positron emission tomography (PET) revealed sex differences in the serotonin neurotransmission.

Major methodological advances and the development of selective radioligands allow now for a precise quantification and localization of serotonergic receptors and the serotonin transporter (Kasper et al. 2002). Using PET and the radioligand α-[^{11}C]methyl-L-tryptophan, the serotonin synthesis rate in the brain of healthy male subjects was found to be higher than the synthesis rate in females (Nishizawa et al. 1997). A lower serotonin transporter binding in women was observed using the radioligand [^{11}C]MADAM (Jovanovic et al. 2007). A lower 5-HT_2 receptor binding capacity in women was also reported (Biver et al. 1996).

Several lines of evidence indicate a sexual dimorphism of the serotonin-1A (5-HT_{1A}) receptor subtype, which is suspected to be substantially involved in the pathogenesis of depressive illness (Drevets et al. 1999), anxiety disorders (Lanzenberger et al. 2007) and suicide (Arango et al. 2001). The 5-HT_{1A} receptor binding is of particular interest for psychiatry as it was shown to correlate with the treatment effect of SSRIs (selective serotonin reuptake inhibitors) as recently demonstrated by our group (Spindelegger, Lanzenberger et al. 2008). The 5-HT_{1A} receptors serve both as somatodendritic autoreceptors on serotonergic neurons in the raphe nuclei of the brainstem and as postsynaptic heteroreceptors. The highest densities of the postsynaptic receptor are found in limbic areas (in particular in the hippocampus and the anterior cingulate cortex), while basal ganglia and the cerebellum exhibit very low densities (Hall et al. 1997). Postsynaptically located 5-HT_{1A} receptors influence a wide range of physiological and behavioral states by modulating cholinergic, dopaminergic, glutamatergic and GABAergic neurotransmitter release (for review, see Fink and Gothert 2007), while autoreceptor activation in the raphe nuclei reduces serotonergic cell firing and inhibits excitation and neural activation in cortical and subcortical target areas (Hajos et al. 2003).

With regard to sex differences, the presynaptic function of the 5-HT_{1A} receptor was proposed to be decreased (Klink et al. 2002) or increased in female rodents (Dominguez et al. 2003). Animal studies suggested area specific sexual dimorphisms with higher postsynaptic 5-HT_{1A} receptor binding in females in some regions (e.g. the anterior cingulate cortex) while lower in other regions, like the hippocampus (Li et al. 2000; Schiller et al. 2006). A higher 5-HT_{1A} receptor density in women was reported post mortem in the dorsal raphe nucleus (Boldrini et al. 2007) and in the prefrontal cortex (Arango et al. 1995). Several other human post mortem studies, however, found no gender differences in binding sites (Palego et al. 1997; Matsubara et al. 1991; Dillon et al. 1991). The few PET studies investigating sex differences in the 5-HT_{1A} receptor in humans *in vivo* resulted in controversial findings. Using the radioligand [*carbonyl*-^{11}C]WAY-100635, either a higher 5-HT_{1A} receptor binding potential in female subjects was reported (Parsey et al. 2002; Jovanovic et al. 2007) or only age related effects without an overall influence of sex (Meltzer et al. 2001).

1.1. OBJECTIVES

Given these controversial results, the aim of the present study was to prove the hypothesis of sex differences in the 5-HT_{1A} receptor binding potential in 32 healthy volunteers (16 male and 16 female subjects) matched to age and socioeconomic status. To account for the presumable influence of gonadal hormones on the receptor binding (Pecins-Thompson and Bethea 1999), all female subjects were measured within the follicular phase of the menstrual cycle. To control for a possible effect of age (Meltzer et al. 2001; Tauscher, Verhoeff et al. 2001), the age range of the subjects was limited to 20-35 years. Furthermore, a newly developed method for the anatomical delineation of the 5-HT_{1A} receptor distribution was introduced using a tracer-specific template, a coregistered region of interest (ROI) template and Automated Anatomical Labelling (Tzourio-Mazoyer et al. 2002).

1.2. HYPOTHESIS

We hypothesized that the 5-HT_{1A} receptor BP_{ND} (the affinity or density of the 5-HT_{1A} receptor) and its distribution in the limbic system differs significantly between healthy men and women.

2. BACKGROUND

2.1. THE SEROTONERGIC SYSTEM

2.1.1. Serotonin – discovery and function

The first notation of 5-hydroxytryptamine (5-HT, serotonin) was made in 1937 (Erspamer and Vialli 1937), but it was not until 1952 that its vasotonic properties made the indoleamine known as "serotonin" (Rapport et al. 1948; Erspamer and Asero 1952). Soon after its discovery in blood serum and in enterochromaffine cells, where 90% of the whole body serotonin can be found, serotonin was identified as a main transmitting substance in the central nervous system (Twarog and Page 1953). In evolutionary terms, it is one of the oldest neurotransmitters (Hen 1993).

Though the number of serotonergic neurons is quite small compared to other neurotransmitter systems (Jacobs and Azmitia 1992), the serotonergic fibers reach all regions of the brain and exert a tonic modulatory influence on a variety of physiological states and behaviors such as the sleep-wake-cycle, sensory-motor control, pain percepetion, appetite, thermoregulation, sexual behavior, cognition and mood (Lucki 1998). Serotonin is furthermore crucially involved in neuronal development and neuroplasticity (Whitaker-Azmitia 2001). The serotonergic system is particularly interesting for psychiatric research as alterations of overall serotonin levels and changes in the serotonergic receptor pattern have been observed in such diverse mental disorders as depression, anxiety, eating disorders, autism, attention deficit disorders or schizophrenia.

2.1.2. Structure and metabolism of serotonin

Serotonin is a biogenic indoleamine with a hydroxy group at the 5 position and a terminal amine group on the carbon chain (figure 1 Nestler et al. 2001). Figure 2.1 shows the structure and metablism of serotonin. As it is charged at physiological pH, it cannot cross the neuronal membrane and must be synthesized within the cytoplasm of serotonergic neurons. The precursor of serotonin, tryptophan, is an essential amino acid actively transported through the blood-brain barrier and into the cytoplasm. In a first step,

tryptophan is converted to 5-hydroxytryptophan, catalyzed by the rate-limiting tryptophan hydroxylase (TPH). In the second step, 5-hydroxytryptophan is converted to 5-hydroxytryptamine by the L-aromatic amino-acid decarboxylase (AADC). Under normal conditions, the tryptophan hydroxylase is about half-saturated. In response to neuronal activation and in the presence of its substrates (tryptophan, oxygen or tetrahydrobiopterin), the TPH activity increases the rate of serotonin synthesis two-fold (Diksic and Young 2001).

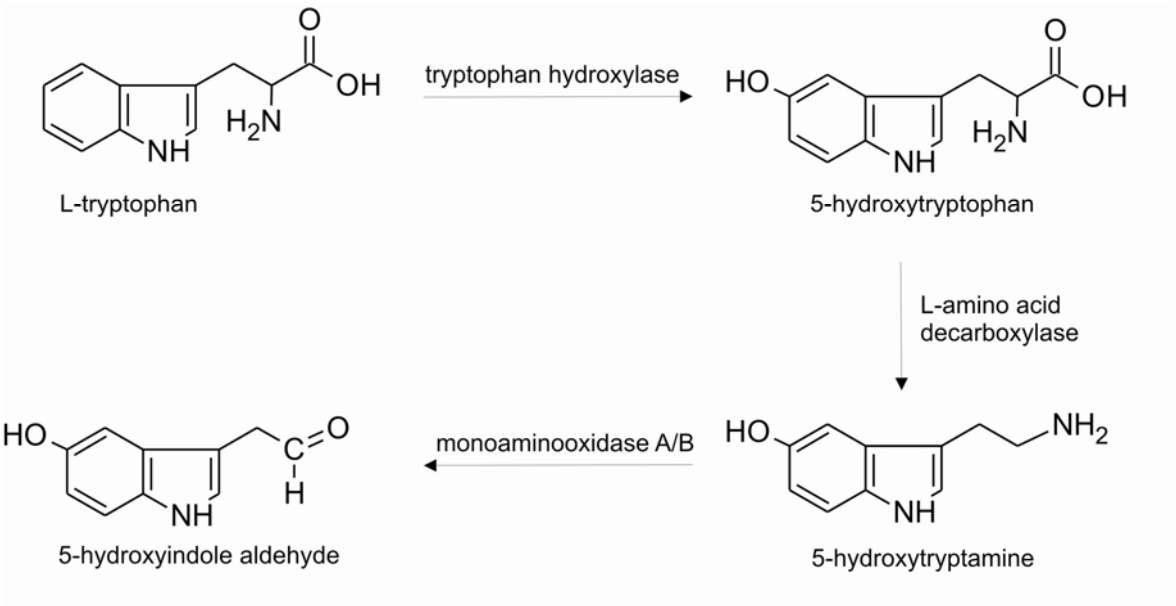

Figure 2.1 Serotonin structure and metabolism

Presynaptically, protected from degradation by a 5-HT binding protein, 5-hydroxytryptamine is transported into storage vesicles via the vesicular monoamine transporter protein (VMAT). Storage vesicles aline along the active zone of the nerve terminal to be released quickly with the influx of Ca^{2+}. With the depolarization of the neuron, the vesicle membrane fuses with the presynaptic plasma membrane and releases stored serotonin into the synaptic cleft. From the synaptic cleft, serotonin exerts its diverse actions binding on at least 16 types of cell surface receptors. The action of serotonin is terminated by the reuptake into the serotonergic nerve terminals via the Na^{+}/K^{+}-dependent serotonin transporter (5-HTT, SERT). Reuptaken serotonin is substrate for the outer membrane mitochondrial enzymes monoaminooxidase A and B. The degradation product 5-hydroxyindole acetic acid (5-HIAA) can be measured in the lumbar cerebrospinal fluid

(CSF) to roughly estimate the brain serotonin turnover. In the pineal gland, serotonin is furthermore converted to melatonin.

2.1.3. Serotonergic pathways

The cell bodies of serotonergic neurons are found largely within the boundaries of the raphe nuclei which are located along the midline of the brainstem. Originally classified in nine clusters B1-B9 (Dahlstrom and Fuxe 1964), the raphe nuclei can be divided into two main groups: the rostral or superior group (B5-B9) extending from the rostral pons to the mesencephalon and the caudal group (B1-B4) extending from the caudal pons to the rostral medulla oblongata. The rostral group accounts for about 85% of the serotonergic neurons and projects formostly to the forebrain and the limbic system. The caudal group, accounting for 15% of the serotonergic neurons, projects into the brainstem and into the spinal cord (Hornung 2003). Figure 2.2 shows the areas innervated by serotonergic fibers ascending from the raphe nuclei.

The rostral group consists of several subnuclei including the median raphe nucleus (MRN, also known as the nucleus centralis superior, B8) and the dorsal raphe nucleus (DRN, B6-B7). Afferent projections to the rostral group are mainly comprised of regulatory glutaminergic neurons from the limbic system and local GABAergic (γ-aminobutyric acid) interneurons. Efferent neurons are extensively arborized and project to all areas of the central nervous system (figure 4 Jacobs and Azmitia 1992). Besides the classic synaptic neurotransmission, serotonin seems to be released in a paracrine manner, which is indicated by the common occurrence of serotonergic varicosities lacking any postsynaptic spezialization (Hensler 2006) and the frequent localization of 5-HT heteroreceptors on non-synaptic sites (Riad et al. 2000).

The slow (1-2 spikes/s) and highly regular pacemaker activity of serotonin is dependent on the waking state and can increase significantly in arousal (Jacobs and Fornal 1999). It is slowed down by the activiation of somatodendritic autoreceptors.

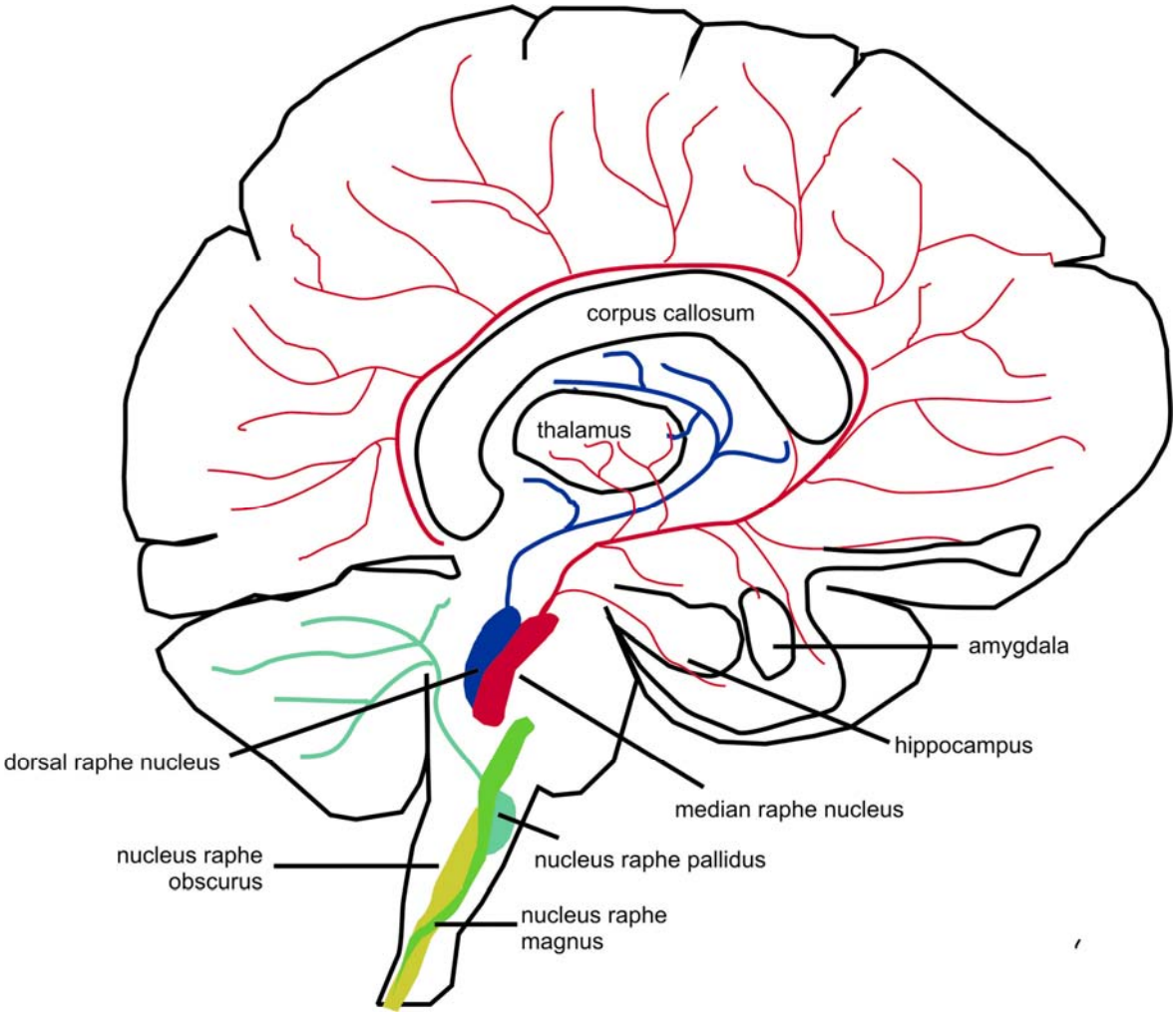

Figure 2.2 Serotonergic pathways (adapted from (Azmitia 2001)

2.1.4. Serotonergic receptors

Serotonergic receptors are classified into seven families, HT_{1-7}, that include at least 16 receptor subtypes with possibly more to be discovered (Niesler et al. 2007). Except for the 5-HT_3 receptors, which are ligand-gated ion channels, all 5-HT receptors are heptahelical, G-protein coupled metabotropic receptors (Barnes and Sharp 1999). It is assumed that the first "primordial" 5-HT receptor evolved over 750 million years ago, which would be prior to the emergence of muscarinic, dopaminergic and adrenergic receptor systems (Peroutka and Howell 1994).

Family	Subtype	Coupled to	Profile
5-HT$_1$	5-HT$_{1A}$	Gi/o, cAMP	somatodendritic autoreceptor, postsynaptic heteroreceptor
	5-HT$_{1B}$	Gi/o, ↓ cAMP	terminal autoreceptor, postsynaptic heteroreceptor
	5-HT$_{1D}$	Gi/o, ↓ cAMP	terminal (+somatodendritic?) autoreceptor, heteroreceptor
	5-ht$_{1E}$	Gi/o, ↓ cAMP	auto- and heteroreceptor?
	5-ht$_{1F}$	Gi/o, ↓ cAMP	auto- and heteroreceptor?
5-HT$_2$	5-HT$_{2A}$	Gq–PLC, ↑ Ca^{2+}	heteroreceptor
	5-HT$_{2B}$	Gq–PLC?, ↑ Ca^{2+}	heteroreceptor
	5-HT$_{2C}$	Gq– PLC, ↑ Ca^{2+}	heteroreceptor, previously classified as 5-HT$_{1C}$
5-HT$_3$	5-HT$_{3A}$	nonselective cation channels	heteroreceptor, also found in autonomic nervous system
	5-HT$_{3B}$		
	5-ht$_{3C}$		
5-HT$_4$		Gs, ↑ cAMP	heteroreceptor
5-ht$_5$	5-ht$_{5A}$	Gi/o?, effect unknown	orphan receptors, predominantly localized on astrocytes
	5-ht$_{5B}$		
5-ht$_6$		Gs, ↑ cAMP	heteroreceptor, found only in the CNS
5-HT$_7$		Gs, ↑ cAMP	heteroreceptor, extensive vascular distribution

Table 2.1 Classification of serotonin receptors (Barnes and Sharp 1999; Hoyer et al. 2002). Gi/o, Gs, Gq – G proteins; cAMP – cyclic adenosine monophosphate; PLC – phospholipase C. Lower case indicates receptors that have not been demonstrated to definitively function in native systems.

5-HT receptors are found on a variety of target cells including motor and pyramidal neurons, Purkinje and granular cells, serotonergic, glutamatergic, GABAergic and cholinergic neurons, neurosecretory neurons, glial, ependymal and endothelial cells (Azmitia 2001). Table 2.1 lists all the known 5-HT receptor subtypes and their profile.

The 5-HT$_1$ receptor class is comprised of five receptor subtypes (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-ht1E, 5-ht$_{1F}$) localized both as autoreceptors on serotonergic cell bodies (mainly 5-HT$_{1A}$) or terminals (mainly 5-HT$_{1B/1D}$) and postsynaptically as heteroreceptors (Barnes and Sharp 1999). The 5-HT$_1$ receptors couple negatively via Gi/o to the adenylyl cyclase resulting in the opening of potassium channels and hyperpolarization of the cell (Hoyer et al. 2002). The former 5-HT$_{1C}$ receptor has been reclassified to 5-HT$_{2C}$ (Baxter et al. 1995).

The 5-HT_2 receptor class consists of three receptor subtypes (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}) which couple to Gq/11 activating phospholipase C. Phospholipase C catalyzes the hydrolysis of phosphatidylinositol into inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 acts on the IP_3 receptor at the endoplasmic reticulum to release Ca^{2+} resulting in the depolarization of the cell (Nestler et al. 2001). Therefore, 5-HT_2 receptors act in an opposing manner to 5-HT_1 receptors. 5-HT_2 receptors can be classified as low-affinity receptors as they need 1000-fold higher serotonin levels than 5-HT_1 receptors to be activated (Azmitia 2001).

5-HT_3 receptors are nonselective cation channels consisting of five subunits surrounding a central ion-conducting pore (Thompson and R. Lummis 2006). They trigger rapid depolarization both in the central and the peripheral nervous system, which is central to the induction of nausea and vomiting. The function of 5-HT_{4-7} receptors has not been clarified yet. While 5-$HT_{4,7}$ and 5-ht_6 couple positively to the adenylyl cyclase, 5-ht_5 receptors (located on astrocytes) are suspected to hyperpolarize the cell by inhibiting the formation of cAMP. The focus of the present study is the 5-HT_{1A} receptor, which is discussed n more detail in the following section.

2.2. THE 5-HT_{1A} RECEPTOR

2.2.1. The 5-HT_{1A} receptor structure

The 5-HT_{1A} receptor is a heptahelical G-protein coupled receptor, with an extracellular amino terminus, seven hydrophobic transmembrane helices connected by three extracellular and three intracellular loops of varying lengths, and an intracellular carboxyl terminus (Kroeze et al. 2002). As described above, it predominantly inhibits the formation of cAMP, which leads to an influx of potassium ions thereby hyperpolarizing the neuron. In the raphe nuclei, the receptor has also been shown to inhibit phospholipase C and Ca^{2+} current and to stimulate the adenylyl cyclase (Clarke et al. 1996).

The human 5-HT_{1A} receptor gene is located on chromosome 5. The encoded protein consists of 422 amino acids and has a core molecular weight of about 46,000 (Raymond et al. 1999).

2.2.2. The 5-HT_{1A} receptor function

The neuronal, neuroendocrine and behavioral effects of the 5-HT_{1A} receptor can be studied by acute or chronic application of 5-HT_{1A} receptor agonists or by blockade of the receptor (see table 2 Passchier and van Waarde 2001). Table 2.2 lists some of the known effects of 5-HT_{1A} agonists.

Acute administration of 5-HT_{1A} agonists induces a variety of cellular and behavioral responses. Activation of 5-HT_{1A} receptors has been demonstrated to inhibit serotonin cell firing (Hajos et al. 2003), glutamate (Matsuyama et al. 1996) and dopamine (Diaz-Mataix et al. 2005) release but to increase the release of noradrenaline (Hajos-Korcsok and Sharp 1996) and acetylcholine (Nakai et al. 1998). The influence on GABA release is unclear (Matsuyama et al. 1996; Kishimoto et al. 2001). 5-HT_{1A} activation furthermore increases the plasma levels of adrenocorticotropin releasing hormone (ACTH), corticosteroids, growth hormone, prolactin and oxytocin (Pitchot et al. 2002; Osei-Owusu et al. 2005; Cleare et al. 1998).

Neurotransmitter	Neuroendocrine	Physiological	Behavioral
Serotonin ↓	ACTH ↑ cortisol ↑	Hypothermia	5-HT behavioral syndrome
Glutamate ↓	Growth hormone ↑	Hyperphagia	Locomotor activity ↑
Dopamine ↓	Oxytocin ↑	Hypotension	Aggressive behavior ↓
Noradrenaline ↑	Prolactin ↑	Bradycardia	Sexual behavior ↑
Acetylcholine ↑	Renin ↑	Bronchoconstriction?	Anxiolysis
GABA ↓↑ ?			Antidepressant action

Table 2.2 Various physiological responses to the administration of 5-HT_{1A} receptor agonists

Physiological responses to 5-HT_{1A} activation include increase in food intake (Curzon 1991), decrease in body temperature (Lerer et al. 1999) and decrease of heart rate and blood pressure (Kubo et al. 1995). Behavioral responses to the 5-HT_{1A} activation include an increase in locomotor activity (Evenden 1994), reduced aggressive behavior (de Boer and Koolhaas 2005) and facilitated sexual behavior (Fernandez-Guasti et al. 1992). 5-HT_{1A} agonists show both anxiolytic and antidepressant properties (Barrett et al. 1994). The "5-HT behavioral syndrome" induced in rats by administration of 5-HT_{1A} receptor agonists is

characterized by several symptoms including hindlimb abduction, forepaw treading, resting tremor, hindlimb rigidity, a "flat body" posture, hyperreactivity, intense salivation, and piloerection (Lucki 1992).

2.2.3. The 5-HT_{1A} receptor distribution and role of regions of interest

The highest densities of 5-HT_{1A} postsynaptic binding sites are found in limbic forebrain regions as in the hippocampus, amygdala, the lateral septum, in cortical areas as the cingulate and entorhinal cortices), in the hypothalamus and in the raphe nuclei (Hall et al. 1997; Varnas et al. 2004). The lowest densities are found in extrapyramidal areas as in the basal ganglia and in the cerebellum, which is frequently used as a reference region. In the raphe nuclei, the 5-HT_{1A} receptors are located presynaptically on serotonergic cell bodies and dendrites. Table 2.3 shows receptor densities in selected regions of interest. The suggested role of these region for emotional regulation is discussed below.

Region	5-HT_{1A} receptor density
Anterior cingulate cortex	+++
Amygdala	BIA + / CeA ++
Insula	+++
Hippcampus	CA1 ++++ CA2,3 +++
Hypothalamus	++
Orbitofrontal cortex	+++
Posterior cingulate cortex	++
Raphe nuclei	+++ / DRN ++++
Retrosplenial cortex	++
Cerebellum	+
Cortical layer	high in I, II low in V,VI

Table 2.3 Receptor densities in selected regions of interest (Hall et al. 1997; Varnas et al. 2004; Luna-Munguia et al. 2005; Schiller et al. 2006). CA1,2,3 – hippocampal areas; CeA – central amygdaloid nucleus; BIA – basolateral amygdala; DRN – dorsal raphe nucleus; + low, ++ moderate, +++ high, ++++ very high density

a. The Anterior Cingulate Cortex (ACC)

The anterior cingulate cortex has been implicated in emotional processing, emotional self-control and problem-solving (Morgane et al. 2005; Etkin et al. 2006; Allman et al. 2001), fear and fear-conditioning (Gao et al. 2004; Pissiota et al. 2003), cognition, learning and memory (Fincham and Anderson 2006) and social skills (Rudebeck et al. 2006). Alterations in the activation pattern of the anterior cingulate have been observed in several psychiatric disorders (Bissiere et al. 2006; Tamminga 2006) including anxiety disorders (Paulus et al. 2004) and posttraumatic stress disorder (Hamner et al. 1999).

b. The Amygdala (AMY)

The amygdala is fundamentally involved in fear, fear conditioning, memory of positive and aversive events and mediation of stress (Morgane et al. 2005; Herman et al. 2005). The amygdaloid nuclei also influence cortical plasticity, attention and cognition (McGaugh et al. 2002; Phelps and LeDoux 2005). Patients with amygdala damage show impaired social behavior accompanied by unusual social fear, deficits in reading facial expressions and troubles with interpreting ambiguous social interactions (Amaral 2002). The lateral nucleus appears to be a "sensory interface" of the amygdala (Phelps and LeDoux 2005), while the central nucleus exerts output and control function (Maren 2001). The amygdala receives input from various regions including the auditory cortex, the thalamus, somatosensory cortex and the hippocampus (Amaral 2002).

c. The Hippocampus (HIP)

The hippocampus ist the main structure of the "hippocampal formation" formed with the entorhinal cortex, the dentate gyrus and the subiculum. It has extensive reciprocal connections both with its surrounding cortical regions as well as with the amygdala, the thalamus and hypothalamus, the anterior cingulate cortex and the association cortices (Amaral and Witter 1989). Based on morphologic and cytoarchitectonic criteria, the hippocampus is divided into the subfields CA1, CA2 and CA3, while CA4 is considered part of the dentate gyrus (Cooper and Lowenstein 2002). The hippocampal formation is essential to episodic and spatial memory formation and consolidation (Martin and Clark 2007). Reduced hippocampal volume has been found in patients with major depression

(Sheline et al. 2002), posttraumatic stress disorder (Tischler et al. 2006), schizophrenia (Steen et al. 2006) and Alzheimer's disease (Devanand et al. 2007).

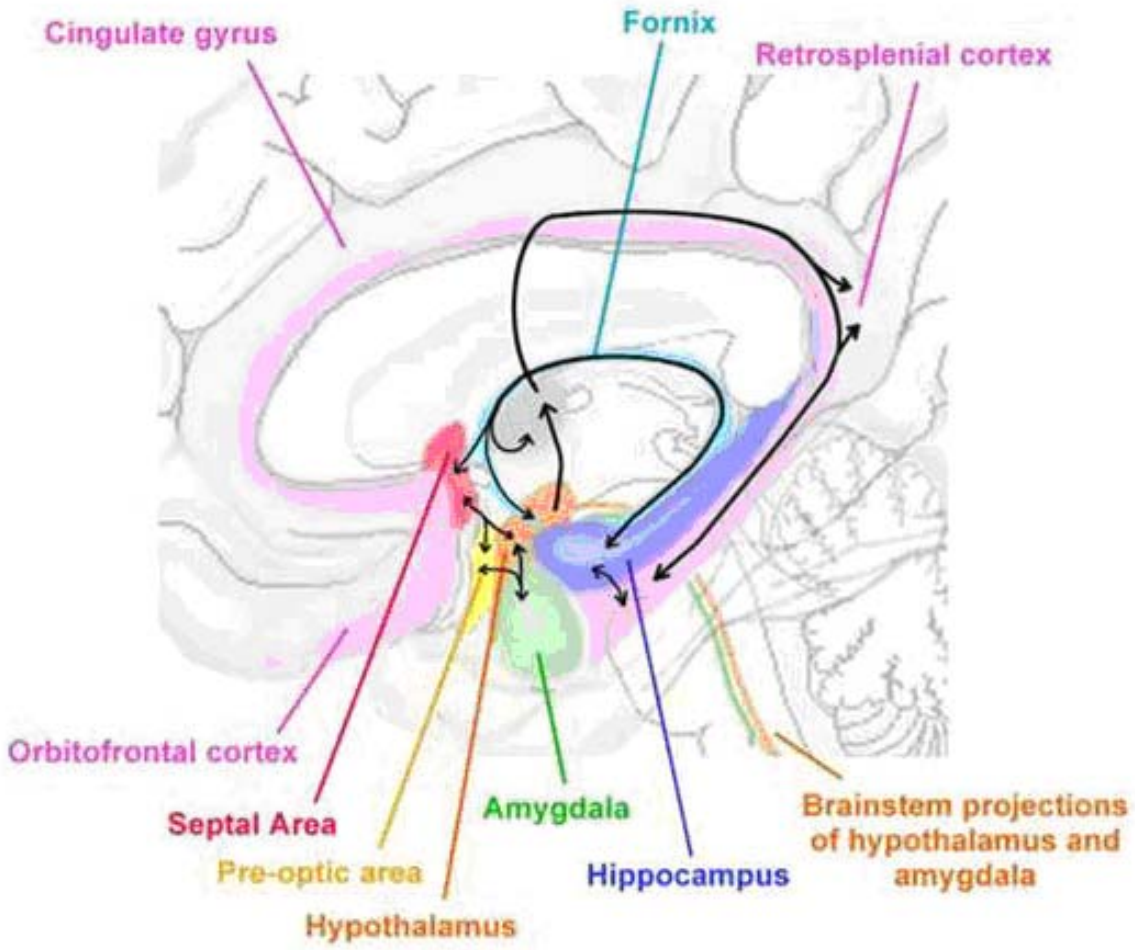

Figure 2.3 Areas of the limbic system. Some of the shown areas were used as regions of interest in the present study (see chapter 3.4). Figure adopted from http://medinfo.ufl.edu/.

d. The Hypothalamus (HYPO)

In its function as the main endocrine regulation center in the central nervous system, the hypothalamus consists of neurons projecting to the anterior and posterior pituitary. The neuronal cell bodies are organized into several nuclei controlling for the release of hormones, thirst and hunger, circadian rhythms and thermoregulation (see review by

Swaab et al. 1993). The hypothalamus contains sexual dimorphic nuclei with an inverted dimorphism in transsexuals (Swaab et al. 2001).

e. The Insular Cortex (INS)

A large part of the insula, which is part of the gustatory cortex, is located in the depth of the lateral sulcus. It is mainly involved in the experience of disgust and experience of aversive stimuli (Wicker et al. 2003). The insula can be divided into two functional sectors: the anterior sector is an olfactory and gustatory center controlling autonomic and visceral function. It is involved in the perception of faces (Keysers and Gazzola 2006) and is probably crucial in the experience and integration of internal emotional state. The posterior part of the insula is connected to auditory, somatosensory, and premotor areas. The insular cortex is bilaterally connected to the amygdala, the nucleus accumbens, the temporal, entorhinal and the orbitofrontal cortex as well as to the anterior cingulate cortex and the thalamus (Paulus and Stein 2006).

f. The Orbitofrontal cortex (OFC)

The orbitofrontal cortex has been demonstrated to be fundamental in making behavioral decisions, particularly in ambiguous and unpredictable situations (Bechara et al. 2000) (Elliott et al. 2000). It has also been linked to reward-related learning and hedonic experience (Kringelbach 2005) as well as to sensory integration, as it receives inputs from a variety of sensory regions like the somatosensory cortex, auditory cortex, olfactory, gustatory and visual cortices and the thalamus (Kringelbach and Rolls 2004). In a reciprocal way, the orbitofrontal cortex is also strongly connected to both the amygdala and the hypothalamus. Further projections go amongst others to the anterior cingulate, the insula, to temporal, retrosplenial and entorhinal cortices, the periaquaeductal grey and the striatum (Cavada et al. 2000). Patients with damage to the orbitofrontal cortex show major changes in emotion, personality, behavior and social interaction (Kringelbach and Rolls 2004). They have troubles decoding social signals as face and voice expression (Hornak et al. 2003).

g. The Posterior Cingulate Cortex (PCC)

The functions of the posterior cingulate cortex have been described as 'evaluative' in contrast to the 'executive' functions of the anterior cingulate cortex. It is essential for visuospatial orientation and self-relevance assessment (Vogt and Laureys 2005).

h. The Retrosplenial Cortex (RSC)

The retrosplenial cortex has been proposed to be involved in episodic memory and in evaluative emotional processing (Maddock 1999). In neuroimaging studies an activation of the retrosplenium was demonstrated when displaying unpleasant emotional auditory or visual (facial) stimuli (Maddock 1999). The retrosplenial cortex receives major inputs from the orbital and dorsolateral prefrontal cortex, the anterior cingulate cortex, temporal and parahippocampal cortex, thalamus and claustrum. It has no direct connections to the amygdala (Vogt and Laureys 2005).

2.2.4. The 5-HT_{1A} receptor in the pathogenesis of mental disorders

Alterations of the 5-HT_{1A} receptor binding potential have been implicated in the etiology of psychiatric disorders. A reduced 5-HT_{1A} receptor binding potential has been consistently observed in depression (Bhagwagar et al. 2004; Sargent et al. 2000; Parsey et al. 2006) and may be predictive to antidepressant treatment response (Meltzer et al. 2004; Spindelegger, Lanzenberger et al. 2008). A reduced 5-HT_{1A} receptor binding was also found in patients with panic disorder (Neumeister et al. 2004) and social phobia (Lanzenberger et al. 2007) as well as in patients with comorbid anxiety (Sullivan et al. 2005), in chronic fatigue syndrome (Cleare et al. 2005) and in Alzheimer's disease (Kepe et al. 2006). Personality traits as anxiety (Tauscher, Bagby et al. 2001) and aggression (Parsey et al. 2002) correlate with a lower 5-HT_{1A} receptor binding potential. On the other hand, bulimia nervosa has been associated with a higher 5-HT_{1A} receptor binding potential during impulsive binge eating (Tiihonen et al. 2004) and subjects with eating disorders were reported to have increased 5-HT_{1A} levels after recovery compared to healthy controls (Bailer et al. 2005). A role of the 5-HT_{1A} receptor in schizophrenia has been implicated but remains controversial (Tauscher et al. 2002; Bantick et al. 2004; Frankle et al. 2006).

2.3. SEX DIFFERENCES IN THE SEROTONERGIC SYSTEM

2.3.1. Rationale for the investigation of sex differences in the serotonergic system

Sexual dimorphisms of the serotonergic system have been reported frequently. In the brain of female rodents, a higher tryptophan content and utilization rate, higher serotonin turnover and serotonin levels have been demonstrated. Several human studies reported a greater responsiveness to serotonergic challenges in female participants. Through major methodological advancements and the development of selective radioligands, *in vivo* assessment of serotonergic structure and function with positron emission tomography also revealed sex differences in the serotonin neurotransmission.

2.3.2. Sex differences in serotonin content

a. Tryptophan

Tryptophan content has been reported consistently higher in female rats (Vaccari et al. 1977; Carlsson et al. 1985) and female mice (Kim et al. 2005) when compared to male rodents. Female rats exert a more pronounced 5-HT behavioral syndrome in response to tryptophan (Carlsson et al. 1985) and the reponse seems to follow shorter latencies and occur with lower doses than in males (Biegon et al. 1979; Dickinson and Curzon 1986). Some human studies reported a higher tryptophan content in the cerebrospinal fluid of healthy women (Young et al. 1980; Anderson et al. 1990) while others did not find any differences (Kennedy et al. 2002). A greater decrease in tryptophan levels was observed in depressed women when compared to depressed men (Anderson et al. 1990; Cowen et al. 1989). Several studies reported stronger effects of tryptophan depletion on mood in women than in men (Sambeth et al. 2007) and lowering of mood after tryptophan depletion has been demonstrated in healthy women without family history of affective disorder (Ellenbogen et al. 1996). The behavioral sex differences are supported by the fact that serotonin synthesis after tryptophan depletion declines to a larger extent in women than in men (Nishizawa et al. 1997). To summarize, though the findings are controverse, a higher tryptophan utilization rate, higher levels of serotonin or a higher synthesis rate in females, as well as a higher serotonergic vulnerability in females were suggested.

b. Overall serotonin levels

Higher serotonin levels in the brain of female rodents when compared to males have been consistently reported (Kato 1960; Dickinson and Curzon 1986; Carlsson et al. 1985; Renner et al. 1985; Borisova et al. 1996) and appear not to be limited to selected regions but to be present in the whole brain (Carlsson and Carlsson 1988). Serotonin synthesis rates were demonstrated higher in female rats (Haleem et al. 1990; Simon and Volicer 1976) and in female monkeys (Young and Ervin 1984). A lower decline in whole brain serotonin levels was observed with age in female rats when compared to males (Weil-Fugazza et al. 1980; Vaccari et al. 1977). In serotonin transporter knock-out mice, a greater increase in serotonin synthesis has been demonstrated in female mice (Kim et al. 2005).

In humans, no sex differences in serotonin availability were found post mortem (Bucht et al. 1981). Using positron emission tomography and the radioligand a-[^{11}C]methyl-L-tryptophan, a higher synthesis in healthy male subjects than in healthy females was observed (Nishizawa et al. 1997; Sakai et al. 2006), while one study described a 10-20% higher synthesis in women (Chugani et al. 1998).

c. Enzyme activity

The activity of the tryptophan hydroxylase in rodents has been demonstrated higher in females than in males (Carlsson and Carlsson 1988), and particularly high in female adult rats (Vaccari et al. 1977). The monoaminooxidase activity might be higher in the cerebral cortex of female rats (Robinson et al. 1971), which fits well into the hypothesis of an overall higher serotonin turnover in females. The enzyme activity also has been reported to fluctuate with the estrous cycle, with highest activity levels positively correlating with progesterone rise (Kamberi and Kobayashi 1970). The prolactin response to fenfluramine (McBride et al. 1990) and meta-chlorophenylpiperazine (Charney et al. 1988; Arato and Bagdy 1998) challenge appears to be higher in female volunteers indicating a higher serotonergic reactivity in females.

d. Five-hydroxyindole acetic acid (5-HIAA)

Levels of 5-HIAA measured in the cerebrospinal fluid (Vaccari et al. 1977; Carlsson and Carlsson 1988) and plasma 5-HIAA levels (Weil-Fugazza et al. 1980) were reported to be

higher in female rats. Furthermore, gender differences in the adaptation of the serotonergic system to stress were shown (Kennett et al. 1986): female rats demonstrated a significant decrease of 5-HIAA levels after repeated stress, which was not observed in male rats. In healthy women, higher 5-HIAA levels were found in the cerebrospinal fluid by one group (Young et al. 1980) but the results were not replicated (Kennedy et al. 2002). Post mortem, lower 5-HIAA levels were reported in male subjects (Bucht et al. 1981).

2.3.3. Molecular imaging of sex differences in the serotonergic system

a. Serotonin transporter (5-HTT, SERT)

[^{3}H]imipramine, a high affinity 5-HTT radioligand, was reported to be higher in female rats (Ieni et al. 1985), but the finding was not replicated in healthy women (Halbreich et al. 1991; Cortes et al. 1988; Soria et al. 1996; Arato et al. 1991). Using [^{3}H]paroxetine, Marazziti et al. investigated post mortem transporter binding sites in female and male subjects. Young females revealed a statistically significant lower binding than males, while the contrary was true for aged females (Marazziti et al. 1998). No sex differences in transporter binding were found in two *in vivo* studies (van Dyck et al. 2000; Best et al. 2005) while one study reported a higher uptake of the ligand in female subjects in the striatum, the brainstem and in the diencephalon (Staley et al. 2001). A recent PET study using the radioligand [^{11}C]MADAM, however, obtained just the opposite result, namely a lower transporter binding in females in all regions investigated (Jovanovic et al. 2007).

b. Serotonin-2A (5-HT$_{2A}$) receptor

The 5-HT$_{2A}$ receptor expression has been shown to vary with estrous cycle in rodents (Sumner and Fink 1997). Sex differences were found in rat hippocampus using [^{3}H]ketanserin (Zhang et al. 1999) and in several regions of the human brain in women using [^{18}F]altanserin (Biver et al. 1996). Two studies using large study samples, however, did not find any sex differences in the 5-HT$_{2A}$ receptor binding in healthy subjects (Versijpt et al. 2003; Adams et al. 2004).

c. Serotonin-1A (5-HT_{1A}) receptor

Animal studies have suggested area specific sexual dimorphisms of the 5-HT_{1A} receptor with higher binding in females in some regions (e.g., the anterior cingulate cortex) while lower in other regions, like the hippocampus (Li et al. 2000). Schiller et al. found lower [^{3}H]8-OH-DPAT binding in female rats in the hippcampus and in the dentate gyrus while reporting higher binding levels in females in the cingulate cortex, the motor cortex and the septum (Schiller et al. 2006). In the juvenile rat brain, Zhang et al. found higher 5-HT_{1A} receptor mRNA levels in the hippocampus, amygdala and in the hypothalamus of female rats but no sex differences in [^{3}H]8-OH-DPAT binding, a 5-HT_{1A} receptor agonist (Zhang et al. 1999). The presynaptic function of the 5-HT_{1A} receptor was proposed to be decreased (Klink et al. 2002) or increased in female rodents (Dominguez et al. 2003).

The first human post mortem studies did not reveal any sex differences in the binding of [^{3}H]8-OH-DPAT (Dillon et al. 1991; Matsubara et al. 1991; Palego et al. 1997). However, an effect of age on the 5-HT_{1A} receptor was suggested as the receptor binding potential declined with age in male but not in female subjects, while a positive correlation between age and the 5-HT_{1A} receptor binding potential in the dentate gyrus, hippocampus and in the parietal cortex was found in females only (Dillon et al. 1991; Palego et al. 1997). A higher 5-HT_{1A} receptor density in women was furthermore reported once in the dorsal raphe nucleus (Boldrini et al. 2007) and once in the prefrontal cortex (Arango et al. 1995).

Four studies have investigated the effect of sex on the 5-HT_{1A} receptor binding potential in humans *in vivo*. While Meltzer et al. found no differences in the mean 5-HT_{1A} BP between sexes, she confirmed the age-related decline of the RBP in male subjects in contrast to females (Meltzer et al. 2001). Other studies suggested an overall higher receptor binding potential in women when compared to men (Parsey et al. 2002; Bhagwagar et al. 2003; Jovanovic et al. 2007).

2.4. POSITRON EMISSION TOMOGRAPHY (PET)

2.4.1. Context for positron emission tomography

Positron emission tomography (PET) allows for the measurement and visualization of the distribution, density and activity of binding proteins, including receptors, transporters and enzymes. PET is the best quantification method for neuronal receptors so far, though it is limited in its anatomical resolution. Magnetic resonance imaging (MRI) delivers precise structural images of the brain in much higher resolution than PET. The combination of both techniques allows for a good localization of the calculated receptor density and affinity.

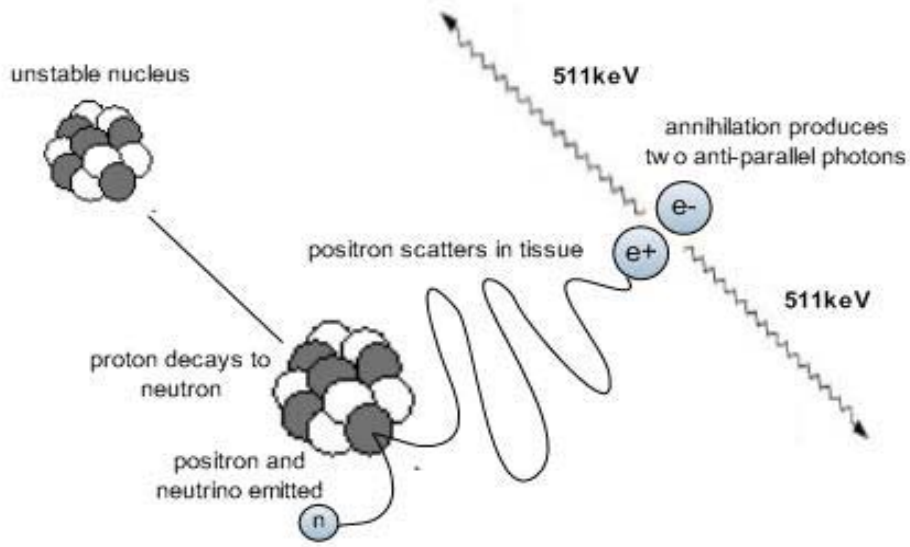

Figure 2.4 Scheme of positron emission: an unstable nucleus decays emitting a positron and a neutrino. The positron travels a short distance in tissue until it collides with an electron. The collision results in annihilation producing two anti-parallel photons (Replicated by courtesy of RD Badawi, Badawi 1998).

2.4.2. Principles of positron emission tomography

PET is based on the chemical properties of proton-rich isotopes that decay when injected into the body. The radioactive tracer travels in the blood stream after injection, penetrating

the blood-brain barrier and spreading out into all brain areas. In the target tissue, the labeled tracer binds to its receptor and accumulates. During its way in the body, the unstable labeling isotope stabilizes its nucleus by converting a proton into a neutron thereby emitting a positron and a neutrino (figure 2.4).

Neutrinos leave the body unaltered and without interaction with tissue. The emitted positron, however, travels only a short distance in the tissue losing energy. At a specific energy level it collides with an electron resulting in the annihilation of both particles. In annihilation, the mass of the electron and positron are converted releasing energy in form of two antiparallel 511keV photons. These gamma rays are emitted at 180-degree to each other. Having high energy, they leave the body without attenuation and may therefore be detected.

2.4.3. The PET Scanner

A PET scanner consists of a full-ring camera that detects emitted gamma rays. Detectors are arranged in one (2D camera) or more (3D camera) rings encircling the body. They are coupled to photomultiplier tubes and connected to an electric circuit. An arriving photon interacts with scintillation crystals of the detector converting into light. The photomultiplier tube notices the light photons and transforms the information into an electrical signal.

Two photons registered simultaneously at facing detectors generate a pulse in the tomograph circuit considered as “coincident” (figure 2.5). The tomograph’s reconstruction software registers the coincidence events at all angular and linear positions of the camera. A straight line estimated between two activated detectors (named "line of response") makes it possible to localize the annihilation event. Summing up all the incoming events leads to a three-dimensional picture showing the radionuclide density in tissue.

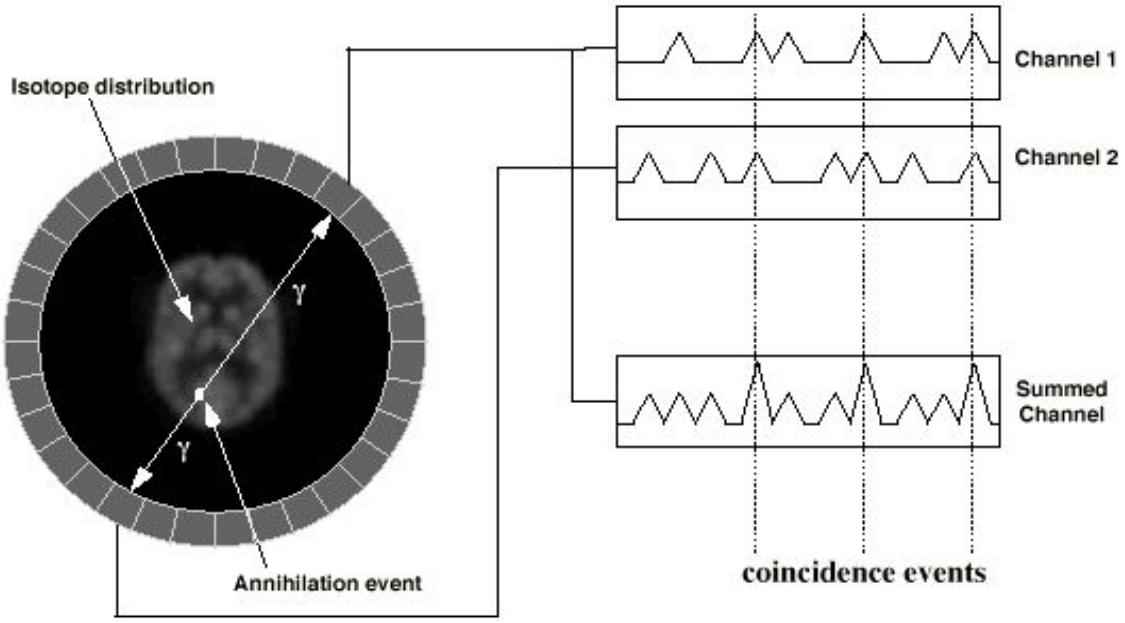

Figure 2.5 The gamma camera: an annihilation event produces two antiparallel photons that are noticed at opposing detectors. Simultaneously occuring signals are considered as coincidence events (Image by Badawi 1999, reprinted with permission from RD Badawi).

Gamma rays that do not coincide are ignored. However, scattered or random coincidences might occur in the same time-window decreasing the contrast and adding noise to the signal. The maximal spatial resolution of PET is determined by the number, efficacy and arrangement of detectors and is limited by the travel distance of photons before annihilation (Cherry and Phelps 1996).

2.5. THE RADIOLIGAND

2.5.1. The development of [*carbonyl*-^{11}C]WAY-100635

The first ligand for PET studies of the 5-HT_{1A} receptor, the ^{3}H labelled 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), was introduced in the 80ties of the 20th century. Though the ligand was highly selective it could not be applied without bias as it caused functional responses by agonistic action at the receptor (Fletcher et al. 1993). WAY100635, N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)N-(2-pyridyl)cyclohexane-carboxamidetrihydrochloride, was developed in the 90ties as the first highly selective

"silent antagonist" at the 5-HT_{1A} receptor (the term "silent" distinguishes pure antagonists with no intrinsic activity from partial agonists, see table 2.4).

Agonists and partial agonists		**Antagonists**	
8-OH-DPAT	Buspirone	Pindolol	WAY 100635
Dipropyl-5-CT	Gepirone	Penbutolol	WAY 100135
LY 274601	Ipsapirone	Tertatolol	Robalzotan
Flesinoxan	Tandospirone	p-MPPI	CPC-222
5-MeODMT	SDZ 216525	p-MPPCl	(S)-UH-301
	MDL 72832	p-MPPF	

Table 2.4 A selection of agonists and antagonists of the 5-HT_{1A} receptor

Shortly after, the first 5-HT_{1A} receptor PET ligand, [O-*methyl*-^{11}C]WAY-100635, was applied in humans (Hume et al. 1994). However, the formation of its radioactive metabolite [O-*methyl*-^{11}C]WAY-100634 caused problems through specific and nonspecific binding in other receptor systems (Cliffe 2000).

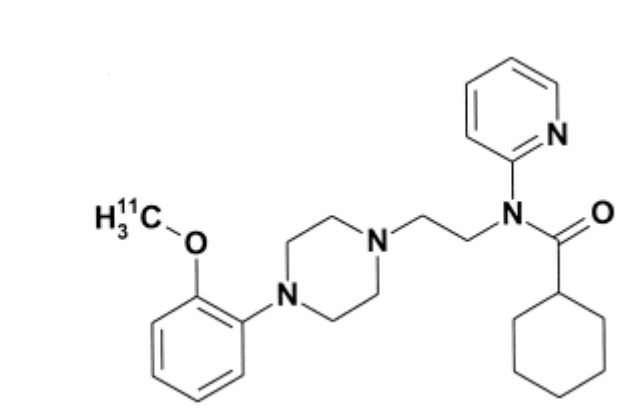

Figure 2.6 Molecular structure of [*carbonyl*-^{11}C]WAY-100635

Therefore, in 1994 a new ligand was introduced, namely [*carbonyl*-^{11}C]WAY-100635 (figure 2.6). The metabolization of [*carbonyl*-^{11}C]WAY-100635 results in its non-radiolabelled metabolite WAY100634 with low crossing of the blood-brain barrier. It was shown that [*carbonyl*-^{11}C]WAY-100635 provides an excellent delineation of 5-HT_{1A} receptors in rat brain (Pike et al. 1995), in rhesus monkey brain (Mathis et al. 1994) and also in humans (Pike et al. 1996). The radioligand appeared to be more selective and had

severalfold greater signal contrast then its predecessors. It had less metabolites and cleared rapidly after injection (Pike et al. 2000; Osman et al. 1998). [*Carbonyl*-^{11}C]WAY-100635 is therefore currently the most frequently used radioligand for the quantification of 5-HT_{1A} receptors with PET.

2.5.2. Radiochemistry of [*carbonyl*-^{11}C]WAY-100635

The PET isotope ^{11}C has a very short half life (20.4 min) and must therefore be produced in a cyclotron directly prior to the measurement. In a cyclotron (figure 2.7), protons are accelerated to collide with a target nucleus producing a proton-rich isotope, e.g. 11-carbon dioxide. The carbon dioxide passes through a cyclotron tube converting into [*carbonyl*-^{11}C]-cyclohexanecarbonyl chloride, the labeling precursor. This precursor can be sealed and heated in a vial containing the target molecule 1-(2-methoxyphenyl)-4-(2-(2-pyridylamino)ethyl)piperazine plus triethylamine. [*Carbonyl*-^{11}C]WAY-100635 is isolated from the vial by sample-enrichment and reverse phase high performance liquid chromatography (HPLC).

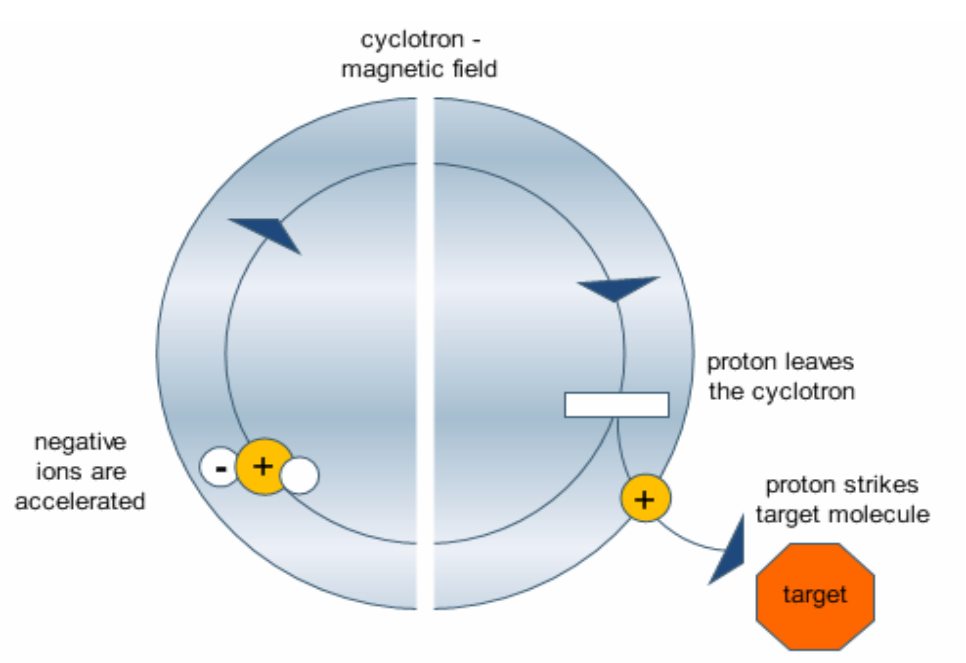

Figure 2.7 Scheme of a cyclotron: a pair of semicircular metal electrodes (“dees”) is placed between the poles of a magnet. The dees induce a high-frequency alternating field that accelerates negatively charged ions. Due to the influence of the strong magnetic field, the charged particles move in a circular path spiraling outward. At a carousel containing thin carbon foils, the positive ion is stripped of the electrons. The remaining positron is directed to a target chamber. In the target chamber, protons can interact with a stable target material to form a radioactive isotope (Image adopted from Cherry and Phelps (2002).

In the present study, the synthesis of [*carbonyl*-^{11}C]WAY-100635 was done with the fully automated PET synthesizer (GE Healthcare, Uppsala, Sweden) at the Cyclotron unit of the PET Centre (Department of Nuclear Medicine at the Medical University of Vienna as described by Wadsak et al. (2007).

For intravenous injection the radioligand was dissoluted in phosphate-buffered saline. The specific activity was determined by HPLC before the injection. It is defined as activity in GBq per amount of substance WAY-100635 in μmol.

2.6. MAGNETIC RESONANCE IMAGING (MRI)

2.6.1. Principles of MRI

The concept of magnetic resonance imaging (MRI) was first introduced in 1952 by Felix Bloch and Edward Purcell. In the last decades MRI has been widely used as a clinical and research tool to obtain structural as well as functional information about the body. Magnetic Resonance Imaging for the generation of structural brain images relies on the properties of hydrogen atoms when placed in a magnetic field.

The spins of hydrogen atoms naturally line up in either the parallel or anti-parallel direction within the main magnetic field (B0) resulting in a parallel net magnetization. Spatial changes of this net magnetization are detectable and can be provoked by applying short electromagnetic radiofrequency (RF) pulses to the body. These pulses are absorbed by the nuclei (excitation) transferring them into a higher energy state (anti-parallel to B0). During the spontaneous realignment of the nuclei into a lower energy state (relaxation), the electromagnetic energy is retransmitted inducing a detectable current in the surrounding coil (the NMR signal). This energy exchange in atoms caused by excitation is called resonance.

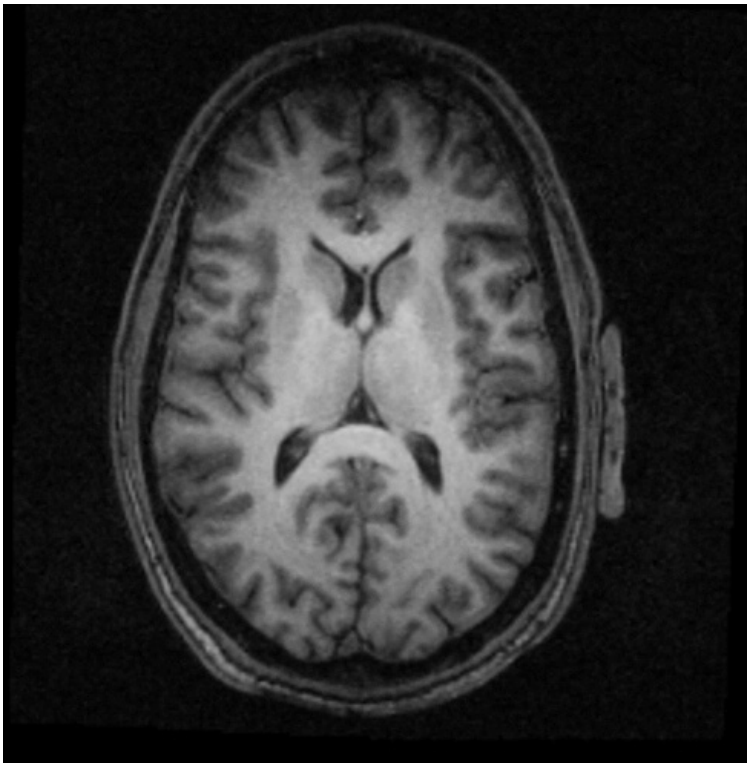

Figure 2.8 A high-resolution, T1-weighted MR image of one subject.

2.6.2. Image reconstruction

The specific proton density of tissue determines its relaxation properties. The relaxation time of the magnetization can be broken down into a longitudinal and a transversal component. Two time constants define the relaxation: after time 1 (T1), the longitudinal magnetization (realignment with B0) has returned to 63 % of its final value, while after time 2 (T2), the transverse magnetization (phase coherence of excited nuclei) has lost 63 % of its original value. T1, T2 and the proton density of the tissue determine the contrast of the image. Finally, spatial information needs to be encoded into the NMR signal. This is done by the employment of magnetic gradients and separate encoding of phase and frequency in distinct slices. The digitized encoded signal is written into a 2-dimensional data matrix called K-space. From the K-space images are reconstructed using a 2D inverse Fourier Transform.

3. METHODS

3.1. OVERVIEW

Eighteen healthy female volunteers and 18 age-matched healthy male volunteers were included in the study. The 5-HT_{1A} receptor BP_{ND} (nomenclature as established by Innis et al. (Innis et al. 2007)) was measured using positron emission tomography (PET) and the highly selective 5-HT_{1A} radioligand [*carbonyl*-^{11}C]WAY-100635. The ability of quantification with PET was combined with the high spatial resolution of magnetic resonance imaging (MRI), which allows for structural mapping of localized binding sites. After the coregistration of the structural MR images to a summation image of dynamic PET scans, regions of interest (ROIs) were delineated manually on the MR images. For automated delineation, a PET template based on Automated Anatomical Labelling (AAL) was created (Tzourio-Mazoyer et al. 2002). Time-activity curves (TACs) of the radioligand in all regions of interest were read out using the software PMOD 2.9. The TACs were fitted using a tracer kinetic model, the Simplified Reference Model (SRTM) with the cerebellum as region of reference, giving a good measure of the respective binding potential in ROIs. An independent samples analysis of variance compared the 5-HT_{1A} BP_{ND} of men and women.

3.2. SUBJECTS

The subjects were recruited from the community via advertisments. To be included in the study, volunteers had to fulfill several criteria. The criteria for participation were age of 20 to 35 years and physical health as assessed by a general physical examination including neurological status, electrocardiogramm and a routine laboratory screening. The narrow age range was chosen to minimize possible age effects on the 5-HT_{1A} receptor BP_{ND} (Tauscher, Verhoeff et al. 2001).

Volunteers with an overall healthy state and no relevant abnormalities were included (see table 3.1). Exclusion criteria comprised any chronic medication or hormonal treatment

including hormonal contraception within 3 months prior to the study, drug abuse, pregnancy, irregular menstrual cycles, abnormalities in the physical examination or any Axis I, DSM IV, psychiatric disorder as assessed by the MINI International Neuropsychiatric Interview obtained by an experienced psychiatrist (Sheehan et al. 1998). Female participants were tested for pregnancy at the screening visit and before each PET measurement using an hCG (human choriogonadotropin) urine test (ACON Laboratories, Inc., San Diego, USA). All subjects gave written informed consent. The study was approved by the Ethics Committee at the General Hospital of Vienna.

Inclusion	Exclusion
Age 20 to 35 years	Medication / hormonal contraception within 3 months prior to the study
Mental health (assessed in psychiatric interview)	History of mental illness or present mental illness
Physical health (assessed in physical examination, laboratory screenings, electrocardiogram, neurological status)	Internistic or neurologic illness
	Drug abuse and history of drug abuse
Females: regular menstrual cycle	Participation in PET or SPECT measurements within 12 months prior to the study
Signed informed consent	Pregnancy

Table 3.1 Inclusion and exclusion criteria

Thirty-six healthy subjects (18 females and 18 males) were included in the study. Female and male subjects were matched for age and socio-economic status. Four subjects were excluded from the final analysis either because of outliers in radiochemical variables (1 female, 1 male), or because the time activity curves of the cerebellar regions exceeded the mean cerebellar binding by more than two standard deviations (Rabiner et al. 2002). See chapter 3.4 for details. The final statistical analysis included 16 female (age 24.1 ± 2.6 years, mean ± SD) and 16 male subjects (age 26.2 ± 4.2 years, mean ± SD). To control for menstrual cycle phase at PET scan, blood samples were collected in the morning prior to the first examination, prior to PET scan and on the day of the final examination from all female participants. Plasma levels of estrogen, progesterone and luteinizing hormone (LH) were quantified by the Clinical Institute for Medical and Chemical Laboratory Diagnostics (KIMCL) at the Medical University of Vienna (for details of standards and references, see chapter 5).

3.3. IMAGE ACQUISITION AND PROCESSING

3.3.1. PET image acquisition

For image acquisition with PET, an Advance high-resolution full-ring PET scanner (General Electric Medical Systems) at the General Hospital of Vienna, Department of Nuclear Medicine, was used. Head movements were minimized by polyurethane molded cushions and straps around forehead and chin.

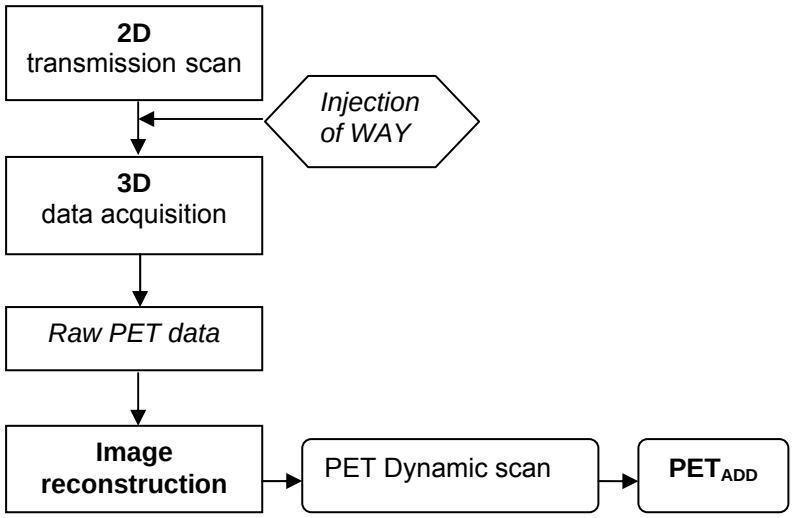

Figure 3.1 Image acquisition diagram – acquisition as described in the main text.

The measurement session (figure 3.1) begun with a five minutes transmission scan in 2D mode. The scan was corrected for attenuation in tissue by using a retractable ^{68}Ge ring source. Transmission scan was followed by the main 3D mode session starting along with the intravenous bolus injection of soluted [*carbonyl*-^{11}C]WAY-100635. A series of 30 time frames (15x1min, 15x5min) was collected in 3D mode resulting in a total acquisition time of 90 minutes.

The acquired raw data were corrected for scatter and random coincidences but not for partial volume. Finally, 35 contiguous slices were reconstructed by an iterative algorithm. The resulting volume (matrix 128*128, slice thickness 4.25mm) had a spatial resolution of 4.36mm full-width half-maximum at the center of the field of view.

3.3.2. Injected dose

The labeled agent, soluted in phosphate-buffered saline (pH 7.4), was injected intravenously right before the 3D mode acquisition scan. The mean injected activity was 5.65 ± 0.79 MBq/kg (mean± SD; n = 32). Specific radioactivity at the time of injection was 152 ± 117 GBq/μmol (mean± SD; n = 32). Injected weight of precursors WAY-100634 and unlabeled WAY-100635 was 2.52 ± 4.5 and 3.3 ± 3.9 μg, respectively. The radioactivity of [*carbonyl*-^{11}C]WAY-100635 was 2.96-5.92 GBq and its radiochemical purity 0.98 ± 0.01%. Sterility, absence of endotoxins, pH, osmolality and residual solvents had been routinely assessed by the PET center.

3.3.3. PET Dynamic scan summation

The final dynamic data consisted of 30 volumes with 35 slices each. With the volume addition tool of PMOD 2.9 all the volumes were added up to one PET summation picture (PET_{ADD}, figure 3.2) in each subject.

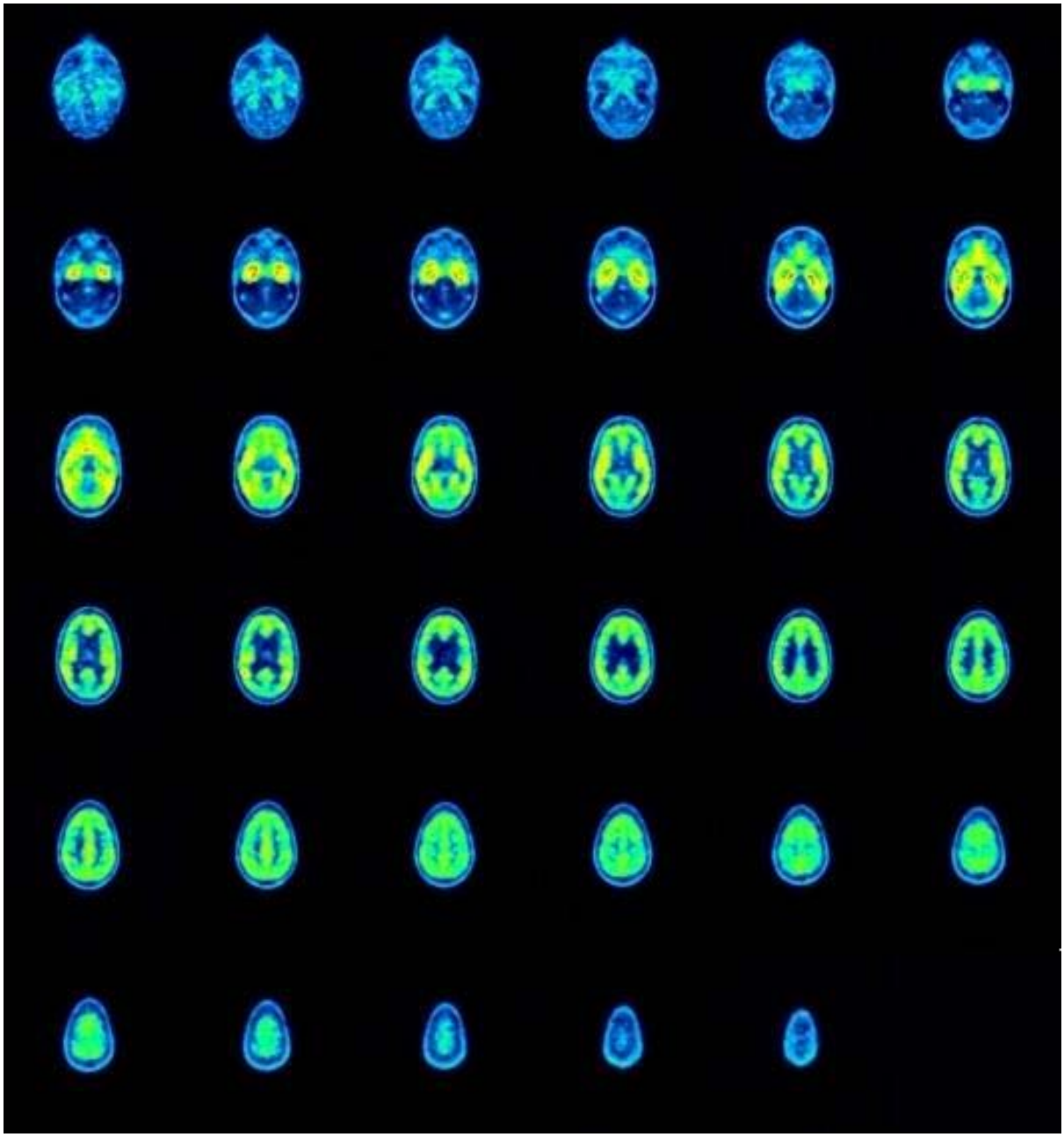

Figure 3.2 Thirty-five slices of the PET summation scan (PET_{ADD})

3.3.4. MR image acquisition

For structural magnetic resonance images, a 3 Tesla Medspec S300 whole-body MR-scanner (Bruker BioSpin, Ettlingen, Germany) was used. High-resolution T1-weighted images were obtained in a 256*256 matrix with a voxel size of 0.78*0.86mm (MPRAGE sequence). Each volume contained 128 slices with a slice thickness of 1.56mm.

3.3.5. Coregistration of MRI images to PET_{ADD}

The individual T1-weighted MR images were coregistered to the PET summation (PET_{ADD}) image using the normalized mutual information method as implemented in the software SPM2 (Friston et al. 1995). Figure 3.3 shows the coregistration procedure.

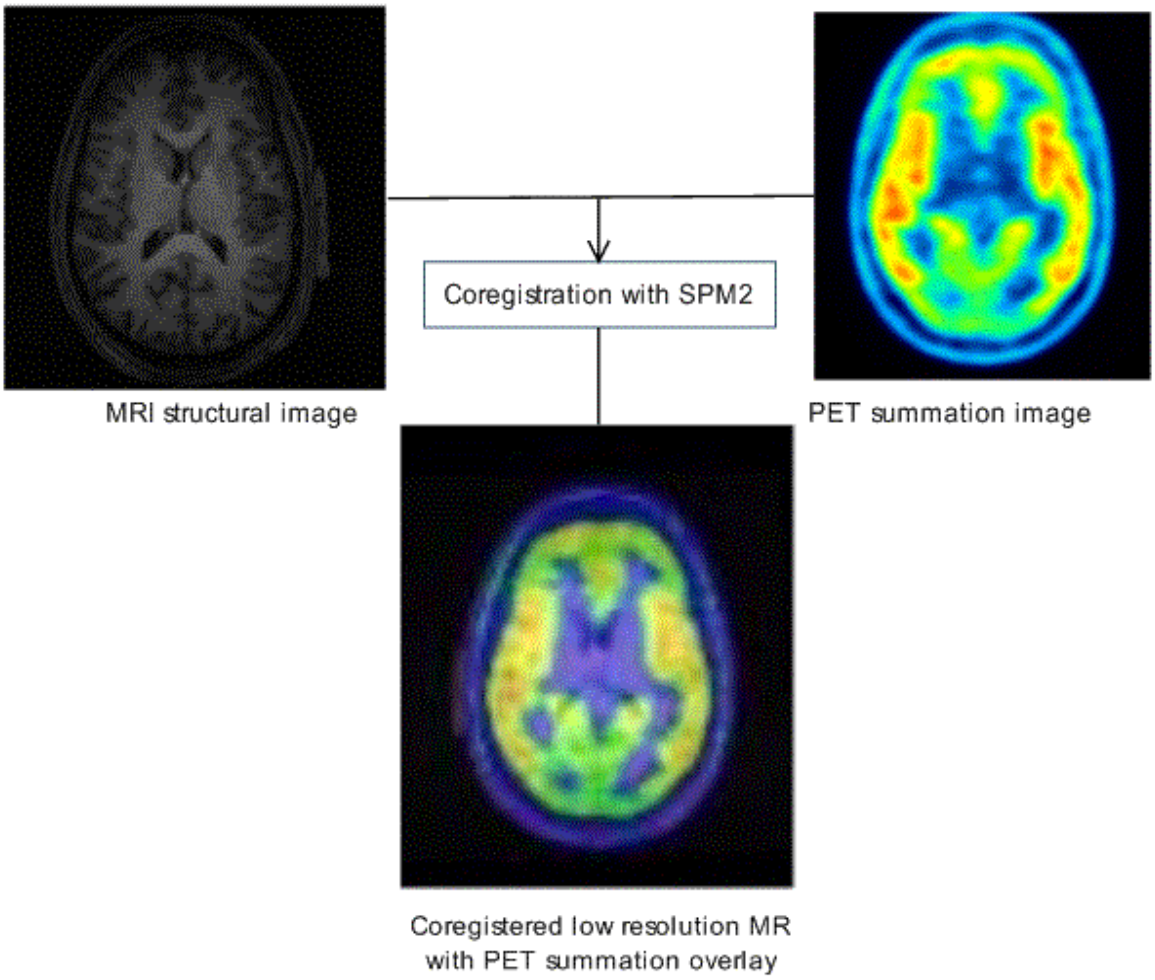

Figure 3.3 Coregistration: A low resolution MR image is coregistered to the PET summation image using SPM2. The PET_{ADD} image can be used as overlay to visualize corresponding regions.

3.4. QUANTIFICATION OF THE BINDING POTENTIAL

3.4.1. Principle

To quantify the 5-HT_{1A} receptor BP_{ND} in regions of interest (ROIs), the kinetic modeling tool of the biomedical image quantification software PMOD 2.9 was used. The ROIs were obtained either by manual delineation on the coregistered low resolution MR images or by automated labelling using a PET template. The 5-HT_{1A} receptor BP_{ND} in distinct regions was calculated using the Simplified Reference Tissue Model (SRTM) with the cerebellum as reference region (Lammertsma and Hume 1996).

3.4.2. Tracer kinetic modeling

Tracer modeling is the mathematical description of radioligand behavior over time. It estimates the delivery of ligand to target tissue, its accumulation and interaction with specific and unspecific binding sites and the clearance from tissue over time. The signal of free, receptor-bound and nonspecifically bound ligand can be differentiated by fitting a mathematical model to the measured and visualized time course of the tracer (time-activity curves, TAC) in a region of interest or voxel (Gunn et al. 2002). Compartment models are widely applied and the basis for the Simplified Reference Tissue approach. Kinetic, equilibrium or graphical methods can be used that either require arterial sampling or not and that describe tracer binding as reversible or non-reversible (Laruelle et al. 2002; Slifstein and Laruelle 2001).

a. The simple model

A compartment is a physiological pool where the radioligand concentration change is assumed to remain homogeneous. The simplest form of a compartment model is the assumption of a direct interaction of ligand with tissue (Ichise et al. 2001). The model compares the concentration of ligand and concentration of available receptors to the concentration of ligand bound to receptor, which is constantly changing. The simple model can be described by the equation $[L]+[R]\leftrightarrow k_{on}/k_{off}\leftrightarrow[LR]$, where [L] is the concentration of radioligand, [R] is the concentration of available receptors and [LR] the concentration of

receptor bound ligand. k_{on} and k_{off} are constants describing the kinetics of association with and dissociation from the receptor.

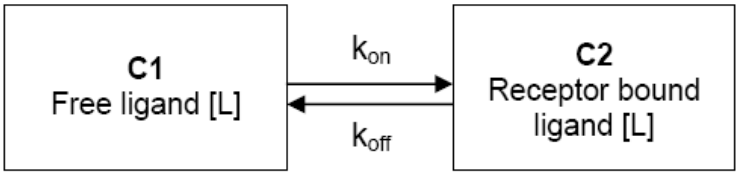

Figure 3.4 Compartment models: scheme of a simple model

Over time, the reaction stabilizes at an equilibrium with a constant dissociation/association from the receptor, defined as K_D (K_D=k_{off}/k_{on}). Given an extremely small radioligand concentration as it is used in PET and a concentration of tissue receptors that nearly equals the total number of receptors (B_{max}), the following equation can be derived: B_{max} / K_D = [RL] / [L]. As BP is defined as B_{max} / K_D we can say that the BP is equal to the ratio of bound radioligand concentration to free radioligand concentration at equilibrium (Ichise et al. 2001) which is equal to B_{max} /affinity of the receptor.

b. The three-compartment model

In PET studies, for the quantification of binding sites, the radioligand is frequently injected intravenously simultaneously with the start of the scan. So at least one more compartment must be assumed than in the simple model, i.e. the first compartment in which the ligand subsides is the arterial plasma. Then, passing the blood-brain barrier, the ligand arrives in the intracellular and interstitial fluid of the brain, which is the second ("free") compartment. The third compartment is the tissue containing receptor with which the ligand interacts. It is important to differentiate receptor-rich regions with specific binding and regions where the radioligand arrives but nearly no specific binding occurs: these can be used as reference region (e.g. the cerebellum, see below).

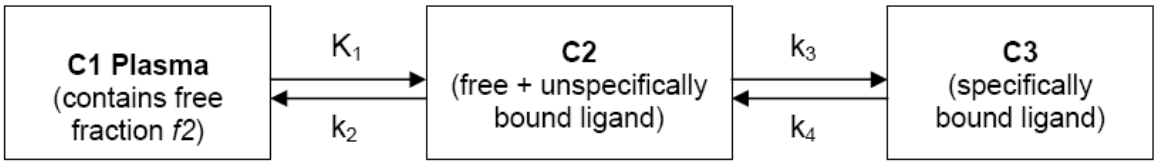

Figure 3.5 Compartment models: scheme of a 3-compartment model

As the non-specifically bound ligand and the free compartment are in a faster exchange than the other compartments, they are considered as one. Classical kinetic models require blood sampling during the measurement to quantify the radioligand and metabolites in plasma. For some tracers a simplified, noninvasive approach turned out to be practicable. It uses a reference region to estimate the input.

c. The Simplified Reference Tissue Model (SRTM)

In 1996 Lammertsma and Hume introduced a three-parameter method to quantify receptors without arterial sampling during the measurements using a noninvasive 2-compartment model.

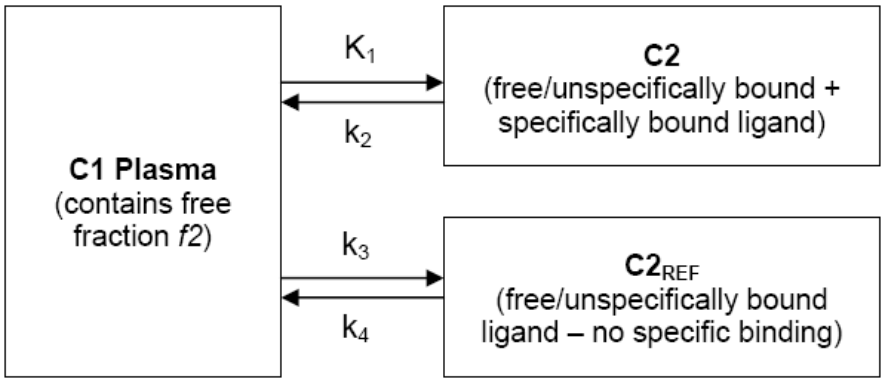

Figure 3.6 The Simplified Reference Tissue Model (2-compartment model with reference region)

The model, known as SRTM (Simplified Reference Tissue Model, figure 3.6), is based on several assumptions: First, it is assumed that the level of free and nonspecific binding of

the radioligand are basically the same in tissue of interest and reference tissue. Second, the exchange between free compartment and compartment of specific binding is so fast that they can be modeled as one compartment. Third, the reference tissue is a region without or with negligibly low specific binding of the radioligand (Gunn et al. 1998; Lammertsma and Hume 1996).

The Simplified Reference Tissue model is based on the following equation:

$$\mathrm{BP} = \frac{B_{\max} f_2}{K_{\mathrm{D}}\left(1 + \Sigma \frac{f_{\mathrm{i}}}{K_{\mathrm{i}}}\right)}$$

where B_{max} is the density of receptors, f_2 is the "free" fraction of the radioligand, K_D is the dissociation constant and f and K are the concentration and dissociation constant of the endogenous radioligand (Rabiner et al. 2002). According to a recent consensus paper (Innis et al. 2007), the nondisplaceble binding potential as quantified using the Simplified Reference Tissue Model is abbreviated as "BP_{ND}".

3.4.3. Delineation of regions of interest

The area-specific binding potential of 5-HT_{1A} receptors was obtained by kinetic modeling using the software PMOD 2.9. Prerequisite for manual delineation was the coregistration of MR and PET images with SPM2 as described above (Meyer et al. 1999). Two approaches for the delineation of regions of interest were applied in this study. The manual method, presently the 'gold standard' method of delineation, defines *a priori* several regions that are individually delineated by the investigator on coregistered MRI images using the anatomical criteria described by Bremner et al. (Bremner et al. 1998).

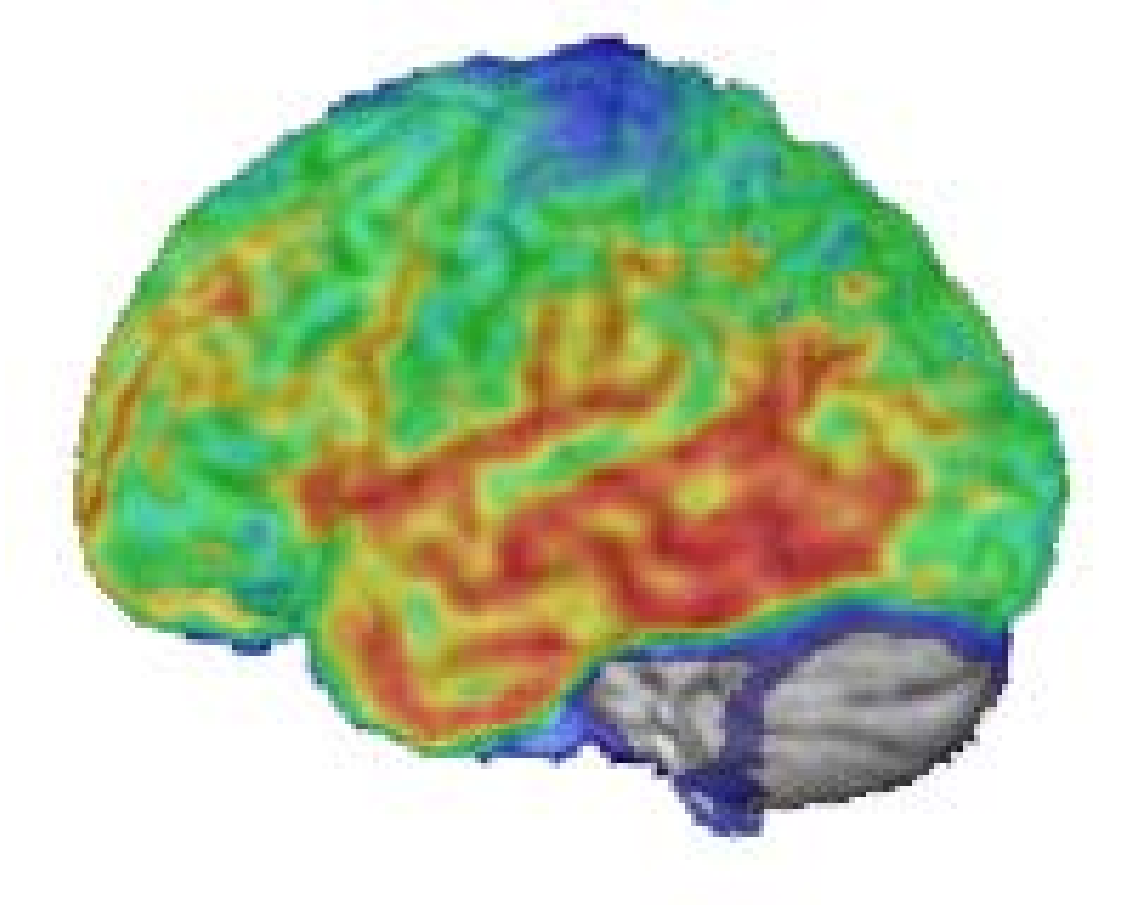

Figure 3.7 A 5-HT_{1A} receptor BP_{ND} distribution map overlayed on a coregistered MR image in one subject. Red → Blue = High binding potential → Low binding potential. Regions of interest were either delineated manually on axial slices of the MR image or a normalized PET ROI template was used (see 3.4.3b).

a. Manual delineation

Nine regions of interest known for their high 5-HT_{1A} receptor density (Hall et al. 1997) were delineated on the coregistered MR images according to the standardized anatomical criteria established by Bremner et al. (Bremner et al. 1998). The regions of interest (given in table 3.2) included the anterior (ACC) and posterior cingulate cortices (PCC), insula (INS), hippocampus (HIP), hypothalamus (HYPO), amygdala (AMY), the medial orbitofrontal cortex (OFC), raphe nuclei (RAPH), the retrosplenial cortex (RSC), and the cerebellum (CER) as region of reference (Burnet et al. 1997). Raphe nuclei were defined on the PET_{ADD} image by fixing a circular volume of interest (VOI, 0.08 cm3) over the highest binding signal in the dorsal midbrain area.

Region	5-HT_{1A} receptor density
Anterior cingulate cortex	+++
Amygdala	++
Insula	+++
Hippcampus	++++
Hypothalamus	++
Orbitofrontal cortex	+++
Posterior cingulate cortex	++
Raphe nuclei	++++
Retrosplenial cortex	++
Cerebellum	+

Table 3.2 The 5-HT_{1A} receptor BP_{ND} in selected regions estimated in autoradiographic studies (Hall et al. 1997): + very low binding potential, ++ moderate, +++ high, ++++ very high receptor binding potential.

The areas were chosen on basis of their relevance for emotional processing and their 5-HT_{1A} receptor densities (see chapter 2.2.3). To be able to delineate the regions of interest on structural images, the individual MR images were coregistered to individual summed PET images (PET_{ADD}) of 30 dynamic time frames using the tool of Statistical Parametric Mapping (SPM2) software (Meyer et al. 1999; Friston et al. 1995).

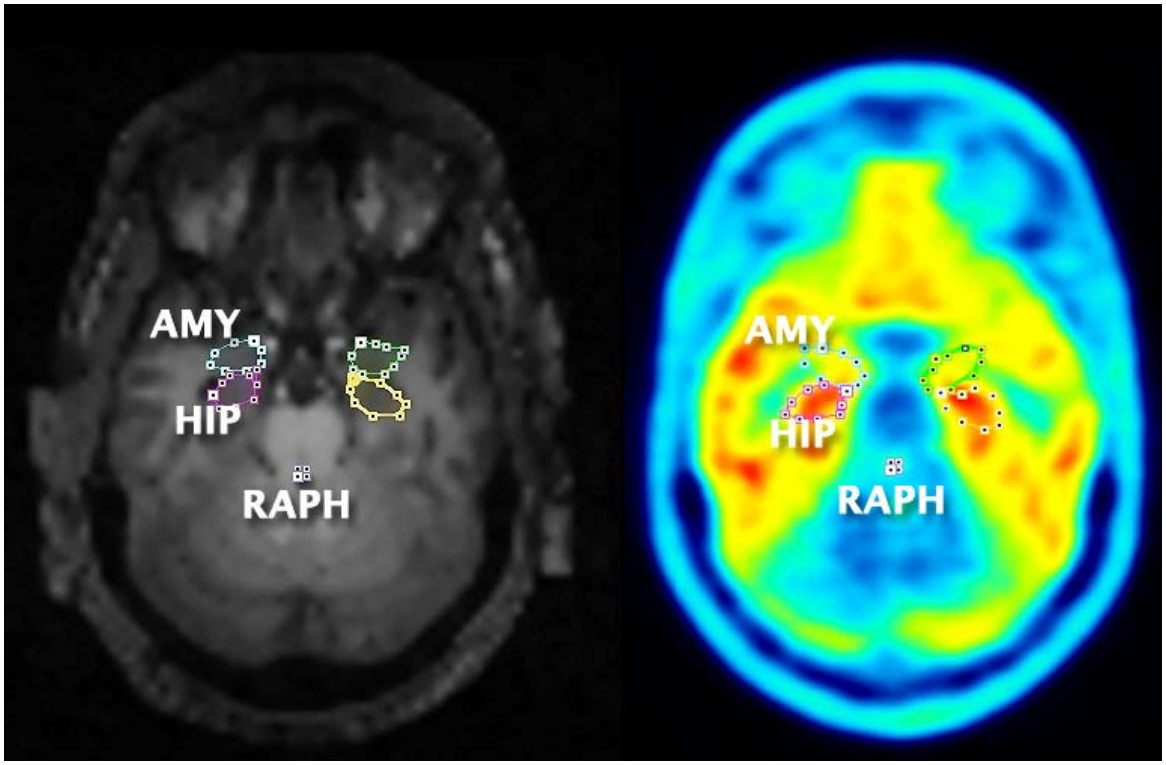

Figure 3.8 ROIs delineated on the coregistered MR-image and ROIs superimposed on the corresponding PET_{ADD} image. AMY – amygdala, HIP – hippocampus, RAPH – raphe nuclei.

The hippocampus (HIP) is bound medially by the perimesencephalic cistern and laterally by the temporal horn. The amygdala (AMY) was identified as the grey matter region directly anterior to the hippocampus. A fixed-size cubic volume of interest for the raphe nuclei (RAPH) was placed on slices showing the interpeduncular cistern. The position of this ROI was drawn according with the highest signal on the corresponding PET_{ADD}. The orbitofrontal cortex (OFC) was situated right above the gyrus rectus in the basal frontal brain. The hypothalamus (HYPO) surrounds the third ventricle. The anterior cingulate cortex (ACC) was delineated on MRI slices in which the caudate, putamen and globus pallidus were clearly seen. The ACC is bound anteriorally by the cingulate gyrus and posteriorally by the corpus callosum. The posterior cingulate gyrus (PCC) was delineated as an area located around the midline of the brain posteriorally to the corpus callosum and surrounded i.a. by the retrosplenium. The insula (INS) lies within the lateral sulcus separating the temporal from the parietal cortex. The retrosplenial cortex (RSC) was found at the most caudal portion of the posterior cingulate cortex (Maddock, 2000).

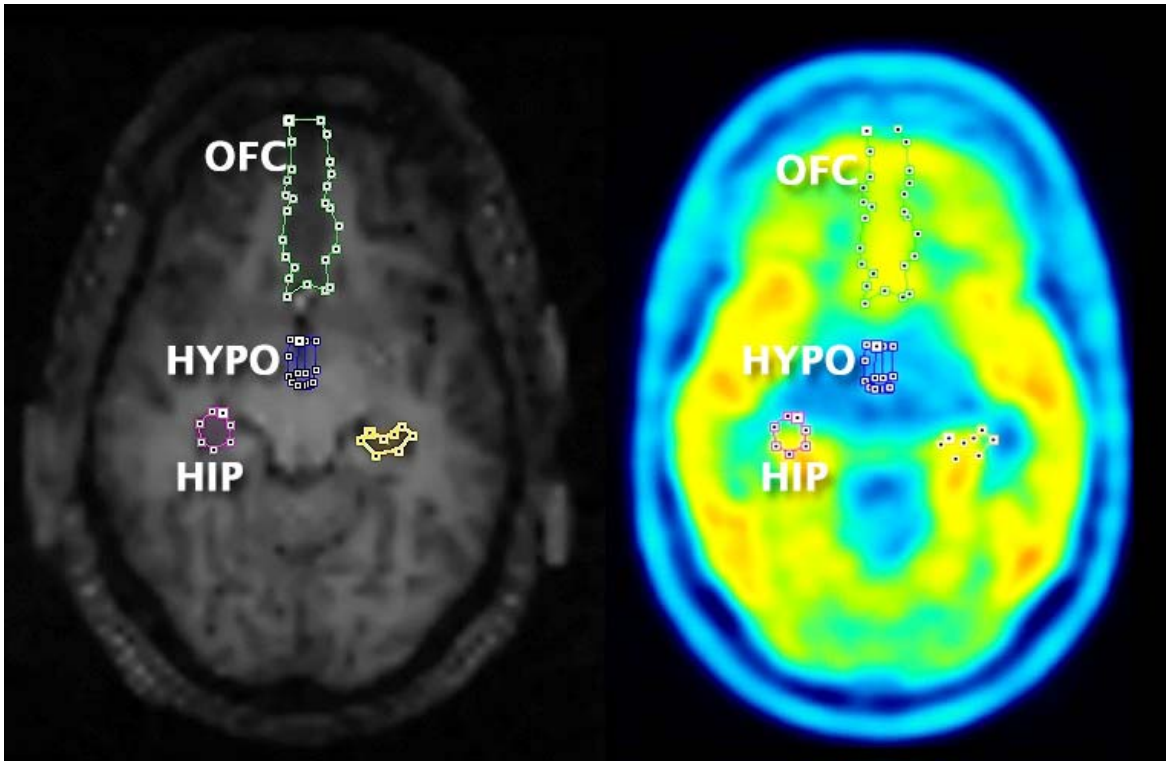

Figure 3.9 ROIs delineated on the coregistered MR-image and ROIs superimposed on the corresponding PET_{ADD} image. OFC – orbitofrontal cortex, HYPO – hypothalamus, HIP – hippocampus.

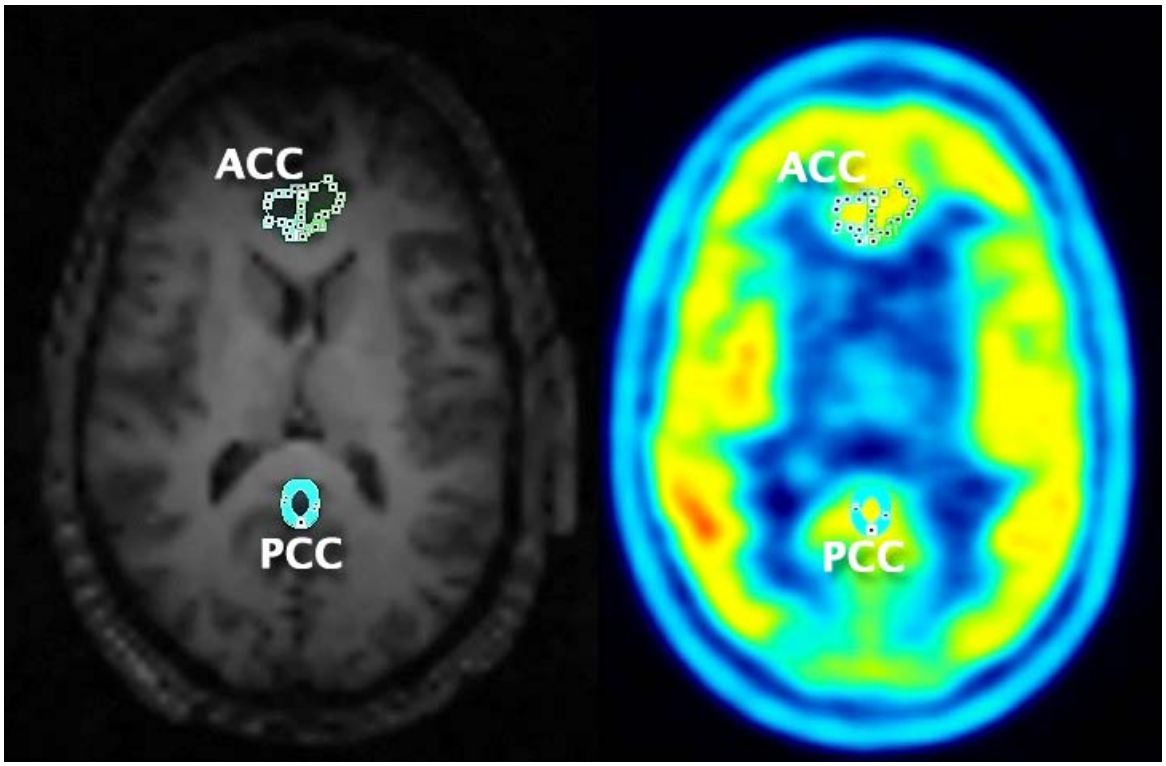

Figure 3.10 ROIs delineated on the coregistered MR-image and ROIs superimposed on the corresponding PET_{ADD} image. ACC – anterior cingulate cortex, PCC – posterior cingulate cortex.

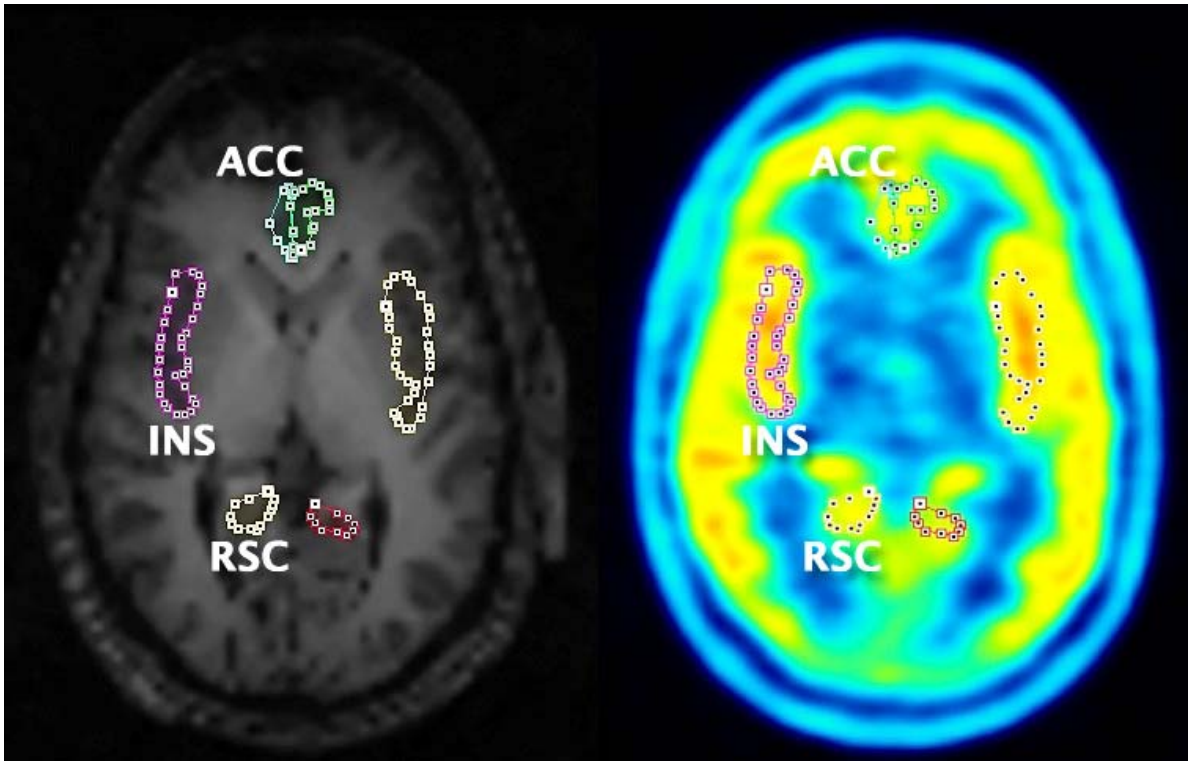

Figure 3.11 ROIs delineated on the coregistered MR-image and ROIs superimposed on the corresponding PET_{ADD} image. ACC – anterior cingulate cortex, INS – insula, RSC – retrosplenial cortex.

b. Automated delineation

For the automated delineation method, we used an ROI template normalized to the 5-HT_{1A} distribution map in the stereotactic space of the MNI/ICBM brain (Montreal Neurologic Institute / International Consortium for Brain Mapping) and PMOD 2.9 (Meyer et al. 1999). Individual dynamic PET data were normalized to the standardized 5-HT_{1A} distribution map that corresponded to the ROI template. All 45 ROIs of this approach were based on the Anatomical Automatic Labelling (AAL) atlas implemented in the SPM2 software (Tzourio-Mazoyer et al. 2002). The ROIs are given in table 3.3. The figures 3.12 and 3.13 show representative ROIs, partly overlayed on the 5-HT_{1A} receptor binding potential map. Time activity curves of the 45 regions were used for quantification in PMOD 2.9.

No.	Region of Interest	No.	Region of Interest
Central region		*Occipital lobe*	
1	Precentral gyrus	25	Superior occipital gyrus
2	Postcentral gyrus	26	Middle occipital gyrus
3	Rolandic operculum	27	Inferior occipital gyrus
Frontal lobe		28	Cuneus
4	Superior frontal gyrus	29	Calcarine fissure
5	Middle frontal gyrus	30	Lingual gyrus
6	Inferior frontal gyrus, opercular	31	Fusiform gyrus
7	Inferior frontal gyrus, triangular	*Limbic lobe*	
8	Superior frontal gyrus, medial	32	Temporal pole: sup. temporal
9	Supplementary motor area	33	Temporal pole: middle temporal
10	Paracentral lobule	34	Anterior cingulate/paracingulate gyrus
11	Superior frontal gyrus, orbital	35	Median cingulate/paracingulate gyrus
12	Middle frontal gyrus, orbital	36	Posterior cingulate gyrus
13	Inferior frontal gyrus, orbital	37	Hippocampus
14	Gyrus rectus	38	Caput hippocampi
15	Olfactory cortex	39	Parahippocampal gyrus
Temporal lobe		40	Insula
16	Superior temporal gyrus	42	Amygdala
17	Heschl gyrus	43	Subgenual anterior cingulate gyrus
18	Middle temporal gyrus	44	Superior frontal gyrus, med.orbital
19	Inferior temporal gyrus	*Raphe nuclei (DRN)*	
Parietal lobe			
20	Superior parietal gyrus		
21	Inferior parietal		
22	Angular gyrus		
23	Supramarginal gyrus		
24	Precuneus		

Table 3.3 The regions of interest used for the automated delineation are based on AAL (Automated Anatomical Labelling) as described by (Tzourio-Mazoyer et al. 2002) and implemented in the SPM2 software. The numbering of the regions corresponds to the ROIs shown in figures 3.12 and 3.13.

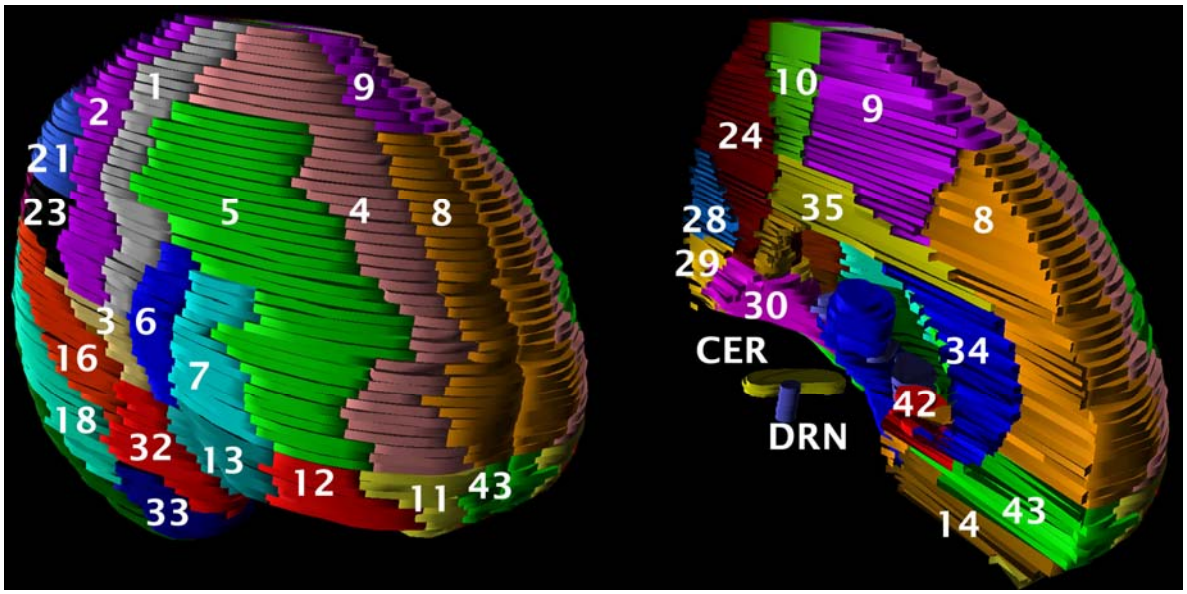

Figure 3.12 A three-dimensional view on the PET template for automated delineation of regions of interest: dorsolateral and sagittal view on the ROI template. CER – cerebellum; DRN – dorsal raphe nuclei. The numbering of the regions corresponds to the regions of interest given in table 3.3.

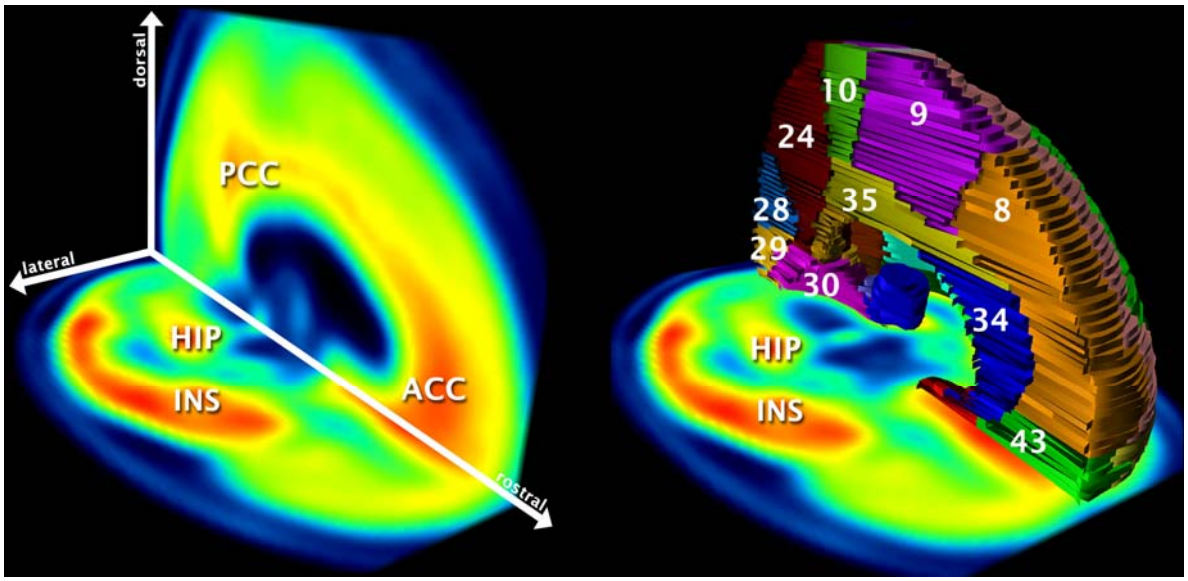

Figure 3.13 A three-dimensional view on the PET template for automated delineation of regions of interest: sagittal and axial views of the serotonin-5-HT_{1A} receptor receptor distribution map. The labelling indicates the localization of regions of interest used for the manual delineation method. ACC – anterior cingulate cortex; PCC – posterior cingulate cortex; HIP – hippocampus; INS – insula. The numbering of the sagittal view on the automated ROI template corresponds to the regions of interest given in table 3.3.

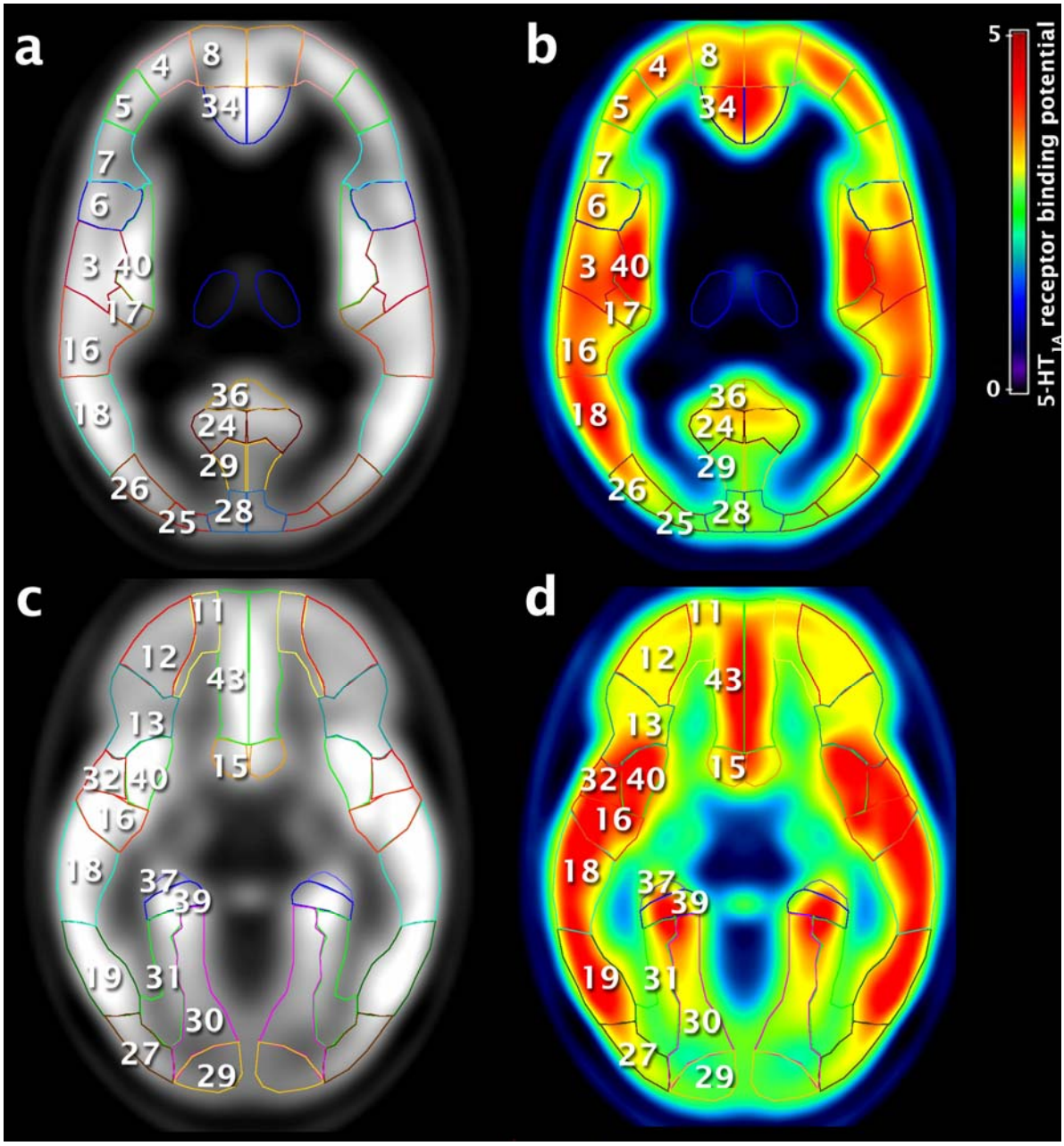

Figure 3.14 The PET template for automated delineation of regions of interest: representative ROIs on two axial slices (a/b vs. c/d) overlayed on a standardized 5-HT_{1A} receptor distribution map either in black and white or in color. Low 5-HT_{1A} receptor BP_{ND} values given in dark blue and high BP_{ND} values given in dark red as indicated in the color table. The numbering of the regions corresponds to the regions of interest given in table 3.3.

c. The cerebellum as region of reference

The rapid metabolization of ^{11}C-labeled tracers makes it difficult to measure the plasma concentration of the ligand and therefore a non-invasive model has several advantages (Slifstein and Laruelle 2001). The Simplified Reference Tissue Model allows to forgo repeated arterial sampling during the PET measure by replacing the arterial input by a reference region. It is known that the cerebellum has a very low 5-HT_{1A} receptor density (Hall et al. 1997; Burnet et al. 1997; Varnas et al. 2004), and a good reliability and reproducibility of using the cerebellum as reference has been demonstrated (Gunn et al. 1998).

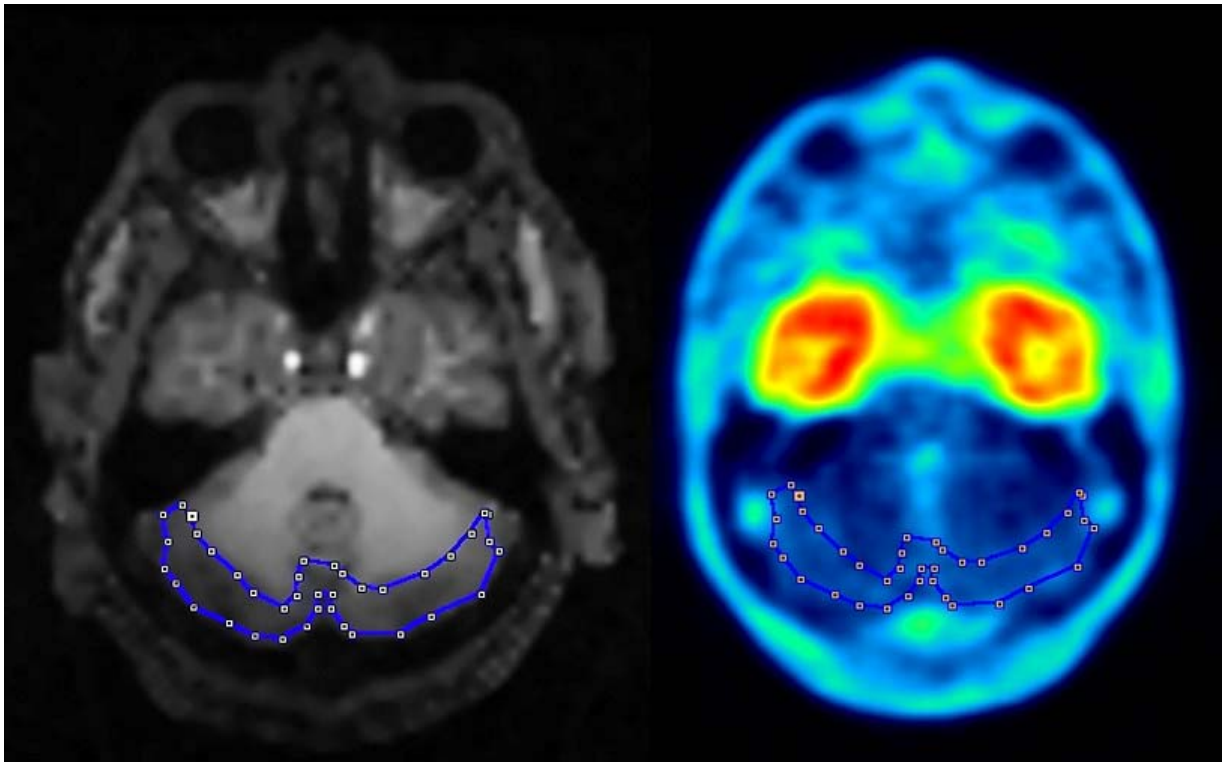

Figure 3.15 ROIs delineated on the coregistered MR-image and ROIs superimposed on the corresponding PET_{ADD} image. The cortex of the cerebellum (CER) is delineated in blue as region of reference.

Recently, possible bias was pointed out as the cerebellar gray matter is not totally devoid of 5-HT_{1A} receptors (Parsey et al. 2005). Though it was suggested that using white matter as reference is more reliable, this may not be appropriate in SRTM analysis due to noise in white matter time activity curves (Hirvonen et al. 2006).

3.4.4. Quantification of the 5-HT_{1A} receptor binding potential

Using the VOI constructor tool of PMOD 2.9, decay-corrected time activity curves (TACs) were generated for each region of interest (figure 3.16 and 3.17) to estimate the time course of the radiotracer. The binding potential was calculated with the kinetic modeling tool of PMOD applying the Simplified Reference Tissue Model by Lammertsma and Hume (1996) as described above.

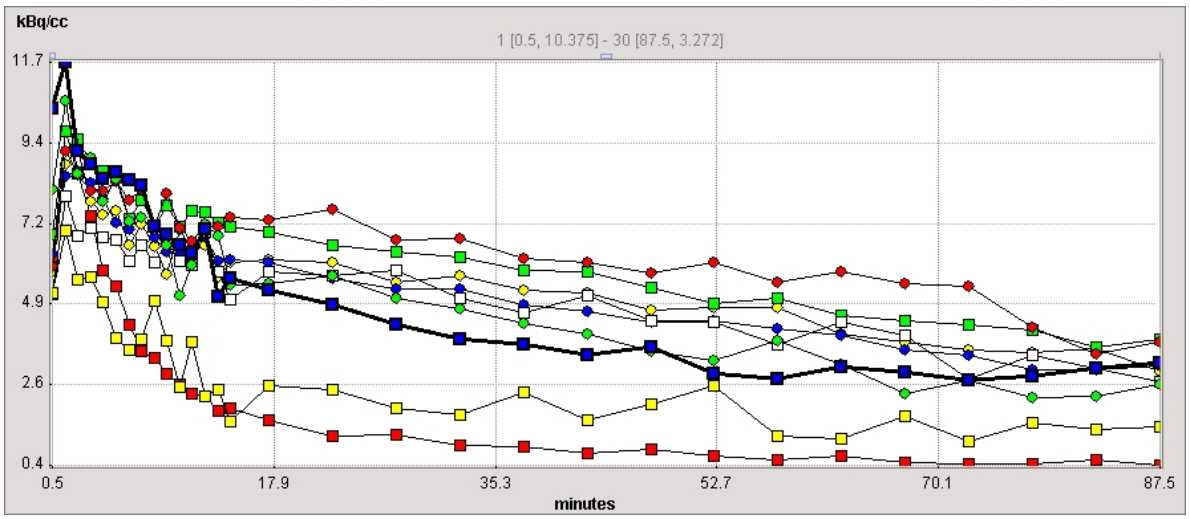

Figure 3.16 Time activity curves of several ROIs in the PMOD kinetic modeling tool. HIP (red circles), OFC (green squares), INS (yellow circles), AMY (white squares), RSC (green circles), PCC (blue squares), ACC (yellow squares), CER (red squares)

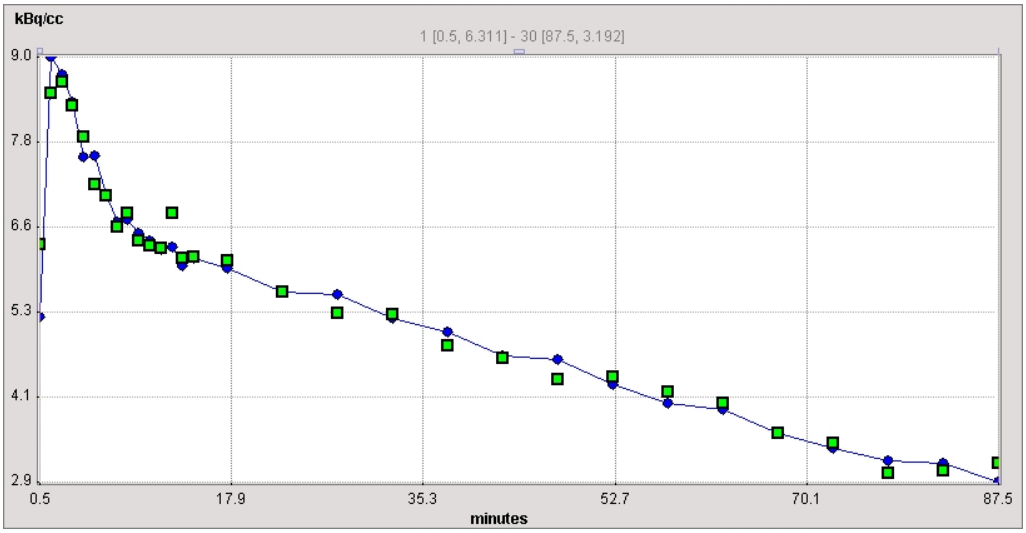

Figure 3.17 Exemplary kinetic SRT model of the receptor binding potential in the orbitofrontal cortex. Green squares: measured time activity of tracer in OFC; blue circles: TAC fitted to calculated model.

3.4.5. Comparison of cerebellar time activity curves (TACs)

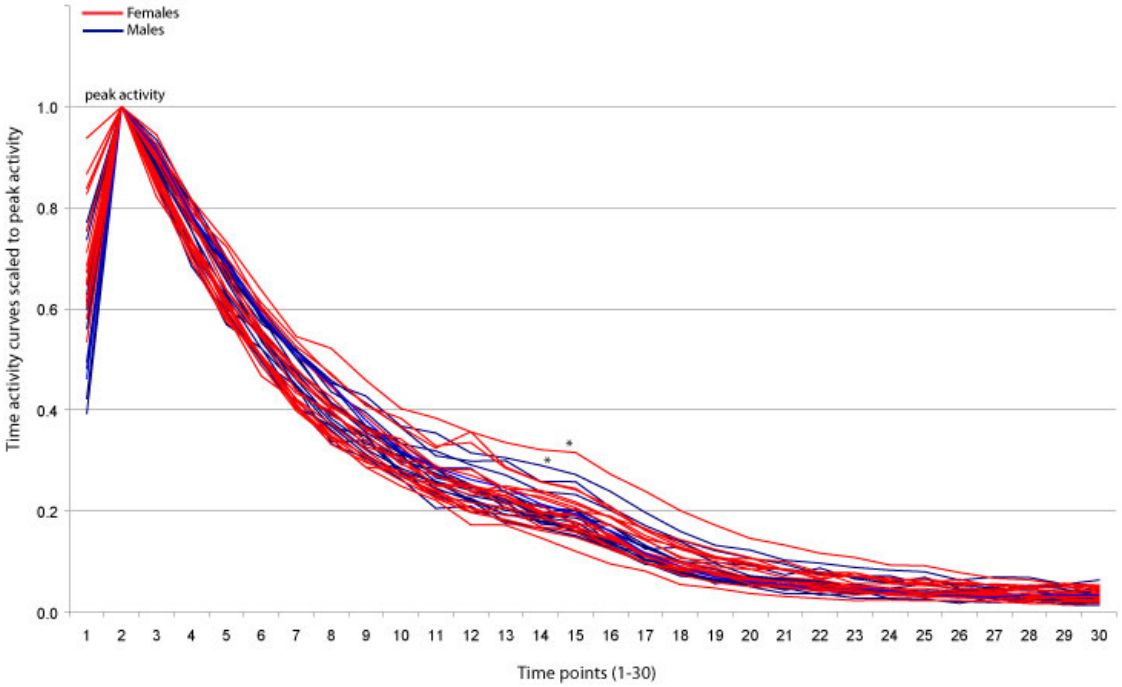

Figure 3.18 Time activity curves of the cerebellum of all participants, plotted to their peak. Red – women, blue – men. The asterisk indicates the TACs of two excluded subjects.

The cerebellar time activity curves of all participants were normalized to their peak. Subjects whose cerebellar TACs exceeded the mean for all subjects by more than two standard deviations in more than two time points were excluded from further analysis. Figure 3.18 shows the TACs of females and males plotted to their peak activity. Based on the higher activity observed in two subjects, one female and one male were excluded from further analysis (asterisk in figure 3.18 indicates the excluded subjects).

3.5. STATISTICAL ANALYSIS

3.5.1. Hypothesis

We hypothesized that the 5-HT_{1A} receptor BP_{ND} (the affinity or density of the 5-HT_{1A} receptor) and its distribution in the limbic system differs significantly between healthy men and women.

3.5.2. Statistical tests

Statistical analyses of the regional mean 5-HT_{1A} receptor BP_{ND} were done using the software SPSS 12.0.1 (SPSS Inc., Chicago, Illinois). The threshold of significance was set at $p \leq .05$, all tests were two-tailed. To control for normal distribution and equality of co-variance, the Kolmogorov-Smirnov test and Levene`s test were performed, respectively. Independent samples *t*-tests were used to test for sex differences in age, radiochemical variables and the normalized regional tracer delivery (R_1). To evaluate a mean effect of sex on the 5-HT_{1A} receptor BP_{ND}, a two-way analysis of variance (ANOVA) was conducted using sex as between-subject factor, region as within-subject factor, subjects as random factor and the interaction term sex by region. A possible effect of age or radiochemical variables on the 5-HT_{1A} receptor BP_{ND} (i.e. injected activity, radiochemical purity and specific activity of the radioligand) was investigated by inclusion of the variables as covariates in the ANOVA. Variables without influence on the 5-HT_{1A} receptor binding potential ($p > .05$) were dropped from further analysis. For an additional, exploratory analysis, independent-sample *t*-tests with sex as independent variable were conducted in each region of interest. Bonferroni adjustment for multiple testing was used to correct for type I error. Both delineation methods were statistically analyzed in the described way.

4. RESULTS

4.1. DESCRIPTIVES AND RADIOCHEMICAL VARIABLES

It has been suspected that the 5-HT_{1A} receptor BP_{ND} may correlate with age and/or body mass index (BMI). The calculation of the 5-HT_{1A} receptor BP_{ND} may also be dependent on the volume of the reference region and may be influenced by radiochemical variables. Regional tracer delivery was furthermore calculated and compared between men and women.

4.1.1. Effects of age, body mass index and radiochemical variables on the 5-HT_{1A} receptor BP_{ND}

The final statistical analysis included 16 female (age 24.1 ± 2.6 years, mean ± SD) and 16 male subjects (age 26.2 ± 4.2 years, mean ± SD). The Kolmogorov-Smirnov test and Levene`s test revealed no significant differences in the equality of distribution or homogeneity of variance between the groups (p>.05). The independent samples t-tests comparing age, body mass index (BMI) and radiochemical variables (radiochemical purity, specific activity and injected dose) in males and females revealed no signficant differences between the groups (p>.05; table 4.1). All tests were two-tailed.

	Males	Females	*t*-test	
	Mean ± SD	Mean ± SD	t	Sig.
Age	26.19 ± 4.20	24.13 ± 2.63	1.67	>.05
BMI	23.19 ± 2.54	22.52 ± 4.21	0.54	>.05
Injected dose (MBq/kg)	5.39 ± 0.59	5.92 ± 0.88	-2.01	>.05

Table 4.1 Mean values for age, body mass index (BMI) and radiochemical variables for men and women (SD – standard deviation). The independent-samples t-tests revealed no significant differences between males and females (p>.05).

4.1.2. Control for menstrual cycle

To control for menstrual cycle phase at PET measurement, blood samples were collected from all female participants in the morning prior to the PET scans. The plasma levels of 17β-estradiol, progesterone and luteinizing hormone (LH) of all female subjects are given in table 4.2. All hormonal levels of the participants were in the reference range for the follicular phase, as given by the Clinical Institute for Medical and Chemical Laboratory Diagnostics (KIMCL) at the Medical University of Vienna

	Mean ± SD	Range	Reference range*
Progesterone (ng/ml)	0.68 ± 0.13	0.46 - 0.86	0.5 - 1.0
17ß-Estradiol (pg/ml)	67 ± 42	20 - 164	22 - 215
Luteinizing hormone (mU/ml)	7.0 ± 2.3	3.6 - 10.3	2.4 - 12.6

Table 4.2 Hormonal plasma levels obtained on the day of PET measurements from female participants. * The reference range applies only to the follicular phase of the cycle.

4.1.3. Control for regional tracer delivery (R_1)

The normalized regional tracer delivery (R_1) values were calculated in all subjects. Table 4.3 shows the R_1 values in males and females in all manually delineated regions of interest. Using both methods, the R_1 values did not differ significantly between men and women (data not shown for the automated method).

	Males	Females	*t*-test	
Region of Interest	**R_1 Mean ± SD**	**R_1 Mean ± SD**	**t**	**Sig.**
Anterior cingulate cortex	0.82 ± 0.06	0.86 ± 0.07	-1.88	0.079
Amygdala	0.69 ± 0.06	0.70 ± 0.04	-0.83	0.413
Hippocampus	0.74 ± 0.06	0.75 ± 0.05	-0.47	0.643
Hypothalamus	0.61 ± 0.08	0.62 ± 0.08	-0.39	0.698
Insula	0.85 ± 0.14	0.90 ± 0.06	-1.19	0.244
Orbitofrontal cortex	0.81 ± 0.05	0.84 ± 0.06	-1.53	0.137
Posterior cingulate cortex	0.96 ± 0.09	1.01 ± 0.10	-1.45	0.156
Raphe nuclei	0.66 ± 0.10	0.72 ± 0.07	-1.84	0.076
Retrosplenial cortex	0.84 ± 0.09	0.85 ± 0.11	-0.12	0.905

Table 4.3 R_1 (regional tracer delivery) values of all manually drawn regions of interest in males and females. The tracer delivery did not differ significantly by sex in any region investigated.

All of the variables (age, BMI and radiochemical variables) were furthermore included as covariates in the independent samples analysis of variance (see chapter 4.3). Variables without influence on the 5-HT_{1A} receptor BP_{ND} (p>.05) were dropped from further analysis.

4.1.4. Regions of interest – volumes

As the same ROI template was used for men and women in the automated approach, only the volumes of the manually delineated ROIs were compared. As indicated in table 4.1, there were no significant volume differences in the manual delineation of ROIs between men and women.

	Males	Females	*t*-test	
Region of Interest - volumes	**Vol Mean ± SD**	**Vol Mean ± SD**	**t**	**Sig.**
Anterior cingulate cortex	1.21 ± 0.06	1.22 ± 0.07	-0.40	0.692
Amygdala	2.59 ± 0.11	2.59 ± 0.08	-0.20	0.847
Hippocampus	2.48 ± 0.09	2.44 ± 0.11	1.24	0.224
Hypothalamus	0.49 ± 0.04	0.47 ± 0.05	0.75	0.459
Insula	9.32 ± 0.84	8.89 ± 0.86	1.45	0.159
Orbitofrontal cortex	7.45 ± 0.88	6.92 ± 0.79	1.78	0.086
Posterior cingulate cortex	1.44 ± 0.07	1.40 ± 0.10	1.23	0.228
Raphe nuclei	0.09 ± 0.02	0.08 ± 0.02	1.01	0.322
Retrosplenial cortex	1.04 ± 0.41	1.07 ± 0.35	-0.19	0.851

Table 4.1 Volumes (in cm^3) of the manually delineated regions of interest in men and women. Independent samples *t*-tests performed in every region of interest revealed no significant difference between the groups.

4.2. RESULTS OF THE MANUAL METHOD

4.2.1. The 5-HT_{1A} receptor BP_{ND} using the manual method

The regional distribution of the 5-HT_{1A} receptor BP_{ND} was in accordance with published *in vivo* and post mortem studies showing the highest 5-HT_{1A} receptor expression in limbic areas as in the hippocampus and the anterior cingulate cortex (Hall et al. 1997). The high inter-subject range in the 5-HT_{1A} receptor BP_{ND} was in accordance with previous reports (Rabiner et al. 2002).

Region of Interest	Males BP Range	Females BP Range
Anterior cingulate cortex	2.37 - 6.15	2.58 - 6.18
Amygdala	2.32 - 5.44	2.17 - 5.04
Hippocampus	2.84 - 8.98	2.98 - 7.47
Hypothalamus	0.62 - 2.31	0.37 - 1.45
Insula	2.99 - 6.89	2.87 - 6.86
Orbitofrontal cortex	2.13 - 5.73	2.34 - 5.46
Posterior cingulate cortex	1.23 - 3.12	1.48 - 3.98
Raphe nuclei	1.08 - 5.29	1.15 - 4.66
Retrosplenial cortex	1.94 - 4.16	1.71 - 3.88

Table 4.2 Range of 5-HT_{1A} receptor BP_{ND} values obtained using the manual delineation method. The high inter-subject variability in the 5-HT_{1A} receptor BP_{ND} is comparable to the observations of other groups (Rabiner et al. 2002).

4.2.2. Regional sex differences in the 5-HT_{1A} receptor BP_{ND} using the manual method

Regional sex differences were investigated by performing independent samples *t*-tests in every region of interest. No significant sex differences were revealed except for a trend in the hypothalamus, were females had a lower 5-HT_{1A} receptor BP_{ND} (t_{30}=2.7; p>.012). This result was not significant as it did not withstand the Bonferroni correction for multiple comparisons with an adjusted p-level of $p \leq .0056$.

	Males	Females	*t*-test	
Region of Interest	**BP Mean ± SD**	**BP Mean ± SD**	**t**	**Sig.**
Anterior cingulate cortex	4.25 ± 1.0	3.94 ± 0.9	0.92	0.366
Amygdala	3.89 ± 0.9	3.52 ± 0.9	1.19	0.245
Hippocampus	5.84 ± 1.9	5.32 ± 1.7	0.83	0.412
Hypothalamus	1.11 ± 0.4	0.77 ± 0.3	2.67	0.012*
Insula	4.77 ± 0.9	4.26 ± 1.0	1.48	0.148
Orbitofrontal cortex	3.60 ± 0.9	3.63 ± 0.8	-0.10	0.921
Posterior cingulate cortex	2.31 ± 0.6	2.32 ± 0.6	-0.03	0.979
Raphe nuclei	2.76 ± 1.1	2.53 ± 0.9	0.63	0.535
Retrosplenial cortex	3.08 ± 0.7	2.89 ± 0.6	0.82	0.420

Table 4.3 Mean regional 5-HT_{1A} receptor BP_{ND} values for men and women (SD – standard deviation) using the manual delineation method. The exploratory independent samples *t*-tests revealed no significant differences between the groups, except for the hypothalamus (* - significant at the *p*-level of .05, uncorrected).

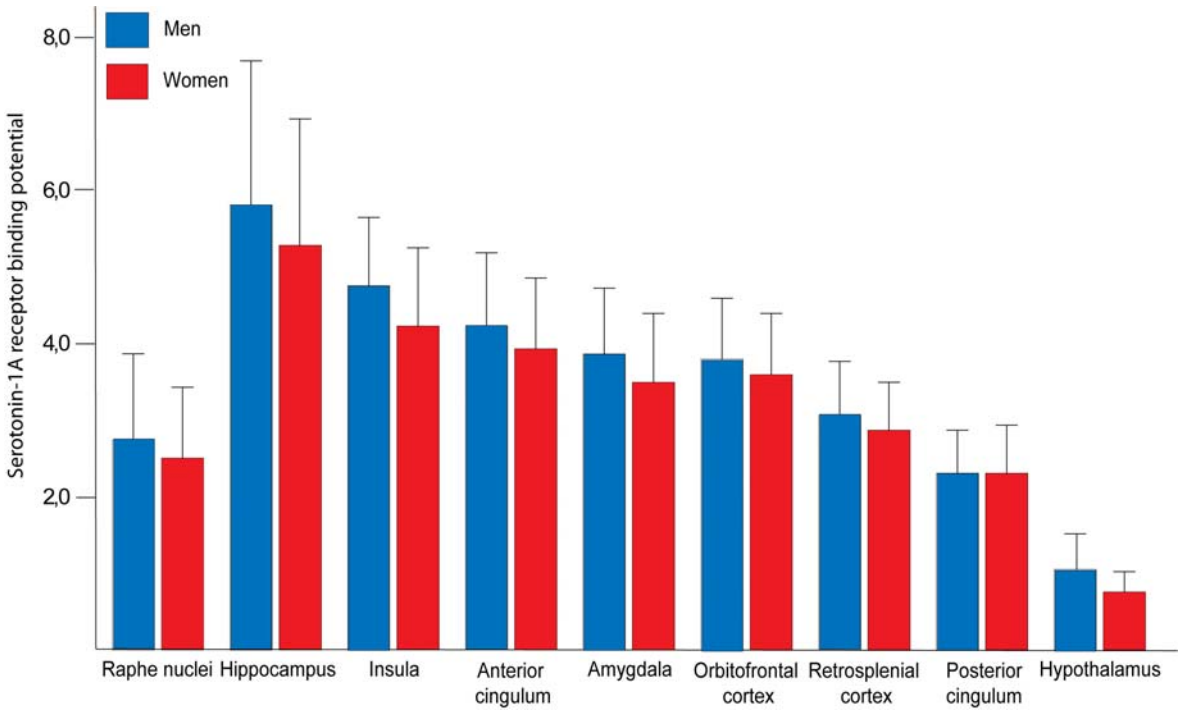

Figure 4.1 Mean 5-HT_{1A} receptor BP_{ND} in men and women using a manual delineation approach in 9 regions of interest and the cerebellum as region of reference (blue bars - men, red bars – women, error bars – standard deviation).

4.2.3. Overall sex differences in the 5-HT_{1A} receptor BP_{ND} using the manual method

An independent samples two-way analysis of variance (ANOVA) was used to compare the 5-HT_{1A} receptor BP_{ND} over all regions of interest. Radiochemical variables and age were first included as covariates, but they were dropped from further analysis as they showed no significant effect on the model (p>.05). Results of the ANOVA are given in table 4.4.

Tests of Between-Subjects Effects			
Fix factor	**df**	***F***	**Sig.**
Sex	1, 30	1.27	0.268
Region	8, 240	127.83	0.000
Sex * Region	8, 240	0.48	0.870

Table 4.4 Two-way ANOVA investigating the influence of sex on the 5-HT_{1A} receptor BP_{ND} in nine regions of interest. The ANOVA reveals a significant effect of region but no effect of sex or the interaction term sex by region. df – degrees of freedom

To conclude, using the manual delineation method, the mean 5-HT_{1A} receptor BP_{ND} over all regions of interest was 3.38 ± 0.07 (mean ± SE) for men and 3.11 ± 0.07 (mean ± SE) for women. The two-way ANOVA did not confirm the hypothesis of a main effect for sex ($F_{1,30}$= 1.3, p=.278). However, a slightly lower mean 5-HT_{1A} receptor BP_{ND} was observed in all regions of interest in females. Also, no significant interaction was found between sex and region. Together with the results from the regional analysis using independent-samples *t*-tests, the results do not support the hypothesis of sex differences in the 5-HT_{1A} receptor BP_{ND} or in the 5-HT_{1A} receptor distribution.

4.3. RESULTS OF THE AUTOMATED METHOD

Consistently, the regional distribution of the 5-HT_{1A} receptor BP_{ND} when using the automated method was in accordance with published *in vivo* and post mortem studies and similar to the manual delineation. It showed the highest 5-HT_{1A} receptor expression in

limbic areas as the hippocampus and the anterior cingulate cortex. The range of the 5-HT_{1A} receptor BP_{ND} in all 45 regions investigated is given in table 4.5 and table 4.6.

4.3.1. The 5-HT_{1A} receptor BP_{ND} using the automated method

		Males	Females
No.	**Region of Interest**	**BP Range**	**BP Range**
Central region			
1	Precentral gyrus	1.88 - 3.3	1.70 - 3.7
2	Postcentral gyrus	1.94 - 3.8	1.79 - 3.8
3	Rolandic operculum	3.01 - 5.9	2.78 - 5.7
Frontal lobe			
4	Superior frontal gyrus	2.31 - 4.3	2.09 - 4.8
5	Middle frontal gyrus	2.54 - 4.5	2.34 - 5.3
6	Inferior frontal gyrus, opercular part	2.40 - 4.8	2.43 - 5.2
7	Inferior frontal gyrus, triangular part	2.25 - 4.1	2.10 - 4.5
8	Superior frontal gyrus, medial	2.31 - 4.6	2.30 - 5.2
9	Supplementary motor area	2.32 - 4.2	2.08 - 4.4
10	Paracentral lobule	1.93 - 4.2	2.02 - 4.1
11	Superior frontal gyrus, orbital part	2.73 - 4.8	2.49 - 5.3
12	Middle frontal gyrus, orbital part	2.62 - 4.8	2.48 - 5.2
13	Inferior frontal gyrus, orbital part	2.39 - 4.5	2.36 - 5.3
14	Gyrus rectus	3.15 - 5.8	2.95 - 7.0
15	Olfactory cortex	2.81 - 6.4	2.16 - 6.6
Temporal lobe			
16	Superior temporal gyrus	2.92 - 5.2	2.52 - 5.7
17	Heschl gyrus	3.06 - 5.4	2.67 - 5.8
18	Middle temporal gyrus	3.16 - 5.6	2.71 - 6.3
19	Inferior temporal gyrus	3.53 - 6.3	2.77 - 6.6

Table 4.5 Range of 5-HT_{1A} receptor BP_{ND} values obtained using the automated delineation method (1/2). The high inter-subject variability in the 5-HT_{1A} receptor BP_{ND} is comparable to the manual delineation results.

No.	Region of Interest	Males BP Range	Females BP Range
Parietal lobe			
20	Superior parietal gyrus	2.22 - 4.3	1.88 - 4.5
21	Inferior parietal, supramarginal/angular	2.48 - 4.5	2.29 - 5.0
22	Angular gyrus	2.75 - 5.0	2.29 - 5.1
23	Supramarginal gyrus	2.87 - 5.3	2.57 - 5.1
24	Precuneus	2.44 - 4.4	2.16 - 4.7
Occipital lobe			
25	Superior occipital gyrus	2.04 - 3.5	1.52 - 4.0
26	Middle occipital gyrus	2.57 - 4.4	2.03 - 5.0
27	Inferior occipital gyrus	2.64 - 4.5	2.22 - 4.7
28	Cuneus	2.06 - 3.4	1.44 - 3.9
29	Calcarine fissure	1.57 - 3.1	1.42 - 3.1
30	Lingual gyrus	2.26 - 3.9	1.77 - 4.1
31	Fusiform gyrus	3.41 - 6.2	3.07 - 6.6
Limbic lobe			
32	Temporal pole: sup. temporal gyrus	3.16 - 6.5	2.90 - 7.3
33	Temporal pole: middle temporal gyrus	3.33 - 6.6	2.78 - 7.4
34	Anterior cingulate/paracingulate gyri	2.50 - 5.1	2.43 - 5.7
35	Median cingulate/paracingulate gyri	2.32 - 4.3	2.12 - 4.8
36	Posterior cingulate gyrus	1.94 - 3.9	1.30 - 3.6
37	Hippocampus	2.34 - 6.8	1.98 - 5.5
38	Caput hippocampi	2.21 - 8.1	2.10 - 7.0
39	Parahippocampal gyrus	3.66 - 7.4	3.42 - 7.6
40	Insula	3.53 - 6.4	3.18 - 7.0
42	Amygdala	2.61 - 5.6	2.42 - 6.1
43	Subgenual anterior cingulate cortex	2.74 - 5.7	1.96 - 5.0
44	Superior frontal gyrus, medial orbital	2.84 - 5.4	2.73 - 5.9
Raphe nuclei (DRN)		1.06 - 5.2	0.59 - 3.4

Table 4.6 Range of 5-HT_{1A} receptor BP_{ND} values obtained using the automated delineation method (2/2). The high inter-subject variability in the 5-HT_{1A} receptor BP_{ND} is comparable to the manual delineation results.

4.3.2. Regional sex differences in the 5-HT_{1A} receptor BP_{ND} using the automated method

As with the manual method, regional sex differences of the 5-HT_{1A} receptor BP_{ND} were investigated by performing independent samples *t*-tests in every region of interest. No significant sex differences were found in any region.

		Males	Females	*t*-test	
No.	**Region of Interest**	**BP Mean ± SD**	**BP Mean ± SD**	**t**	**Sig.**
Central region					
1	Precentral gyrus	2.63 ± 0.4	2.51 ± 0.5	0.71	0.482
2	Postcentral gyrus	2.94 ± 0.5	2.74 ± 0.5	0.99	0.328
3	Rolandic operculum	4.50 ± 0.9	4.22 ± 0.8	0.94	0.356
Frontal lobe					
4	Superior frontal gyrus	3.32 ± 0.5	3.22 ± 0.7	0.46	0.649
5	Middle frontal gyrus	3.51 ± 0.6	3.41 ± 0.7	0.43	0.667
6	Inferior frontal gyrus, opercular	3.66 ± 0.6	3.50 ± 0.7	0.68	0.501
7	Inferior frontal gyrus, triangular	3.25 ± 0.5	3.17 ± 0.6	0.40	0.693
8	Superior frontal gyrus, medial	3.56 ± 0.6	3.48 ± 0.7	0.33	0.744
9	Supplementary motor area	3.19 ± 0.5	3.06 ± 0.6	0.63	0.533
10	Paracentral lobule	3.09 ± 0.6	3.00 ± 0.6	0.44	0.664
11	Superior frontal gyrus, orbital	3.76 ± 0.6	3.63 ± 0.7	0.53	0.597
12	Middle frontal gyrus, orbital	3.69 ± 0.7	3.48 ± 0.7	0.84	0.406
13	Inferior frontal gyrus, orbital	3.62 ± 0.6	3.50 ± 0.8	0.49	0.628
14	Gyrus rectus	4.70 ± 0.8	4.54 ± 1.1	0.47	0.639
15	Olfactory cortex	4.67 ± 1.1	4.42 ± 1.1	0.64	0.527
Temporal lobe					
16	Superior temporal gyrus	4.25 ± 0.7	4.02 ± 0.8	0.83	0.414
17	Heschl gyrus	4.22 ± 0.8	4.00 ± 0.8	0.81	0.427
18	Middle temporal gyrus	4.57 ± 0.8	4.27 ± 0.9	0.99	0.331
19	Inferior temporal gyrus	5.01 ± 0.9	4.60 ± 1.0	1.26	0.219

Table 4.7 Mean regional 5-HT_{1A} receptor BP_{ND} values for men and women (SD – standard deviation) using the automated delineation method (1/2). The exploratory independent samples *t*-tests revealed no significant differences between the groups in any region.

		Males	Females	*t*-test	
No.	**Region of Interest**	**BP Mean ± SD**	**BP Mean ± SD**	**t**	**Sig.**
Parietal lobe					
20	Superior parietal gyrus	3.11 ± 0.6	3.02 ± 0.7	0.40	0.693
21	Inferior parietal	3.47 ± 0.6	3.41 ± 0.7	0.26	0.799
22	Angular gyrus	3.70 ± 0.6	3.57 ± 0.7	0.53	0.597
23	Supramarginal gyrus	4.05 ± 0.7	3.80 ± 0.7	1.00	0.326
24	Precuneus	3.36 ± 0.6	3.27 ± 0.6	0.41	0.682
Occipital lobe					
25	Superior occipital gyrus	2.84 ± 0.5	2.68 ± 0.6	0.86	0.395
26	Middle occipital gyrus	3.57 ± 0.6	3.37 ± 0.7	0.87	0.391
27	Inferior occipital gyrus	3.67 ± 0.6	3.42 ± 0.7	1.10	0.278
28	Cuneus	2.77 ± 0.4	2.62 ± 0.6	0.77	0.448
29	Calcarine fissure	2.47 ± 0.5	2.33 ± 0.4	0.91	0.372
30	Lingual gyrus	3.17 ± 0.5	2.98 ± 0.6	1.00	0.328
31	Fusiform gyrus	4.98 ± 0.9	4.62 ± 0.9	1.13	0.266
Limbic lobe					
32	Temporal pole: sup. temporal	5.11 ± 1.0	4.77 ± 1.1	0.96	0.344
33	Temporal pole: middle temporal	5.27 ± 0.9	4.91 ± 1.2	0.98	0.333
34	Anterior cingulate/paracingulate	4.02 ± 0.7	3.72 ± 0.9	1.01	0.318
35	Median cingulate/paracingulate	3.33 ± 0.6	3.18 ± 0.7	0.62	0.537
36	Posterior cingulate gyrus	2.80 ± 0.6	2.47 ± 0.6	1.57	0.127
37	Hippocampus	4.51 ± 1.4	4.18 ± 1.0	0.77	0.448
38	Caput hippocampi	5.04 ± 1.8	4.62 ± 1.3	0.76	0.454
39	Parahippocampal gyrus	5.73 ± 1.1	5.33 ± 1.2	0.98	0.337
40	Insula	5.12 ± 0.9	4.76 ± 1.0	1.08	0.290
42	Amygdala	4.41 ± 1.0	4.06 ± 1.0	0.98	0.334
43	Subgenual anterior cingulate	3.97 ± 1.0	3.63 ± 0.9	1.05	0.304
44	Superior frontal gyrus, med.orbital	4.19 ± 0.8	4.03 ± 0.8	0.59	0.562
Raphe nuclei (DRN)		1.98 ± 1.0	1.65 ± 0.7	1.09	0.286

Table 4.8 Mean regional 5-HT_{1A} receptor BP_{ND} values for men and women (SD – standard deviation) using the automated delineation method (2/2). The exploratory independent samples *t*-tests revealed no significant differences between the groups in any region.

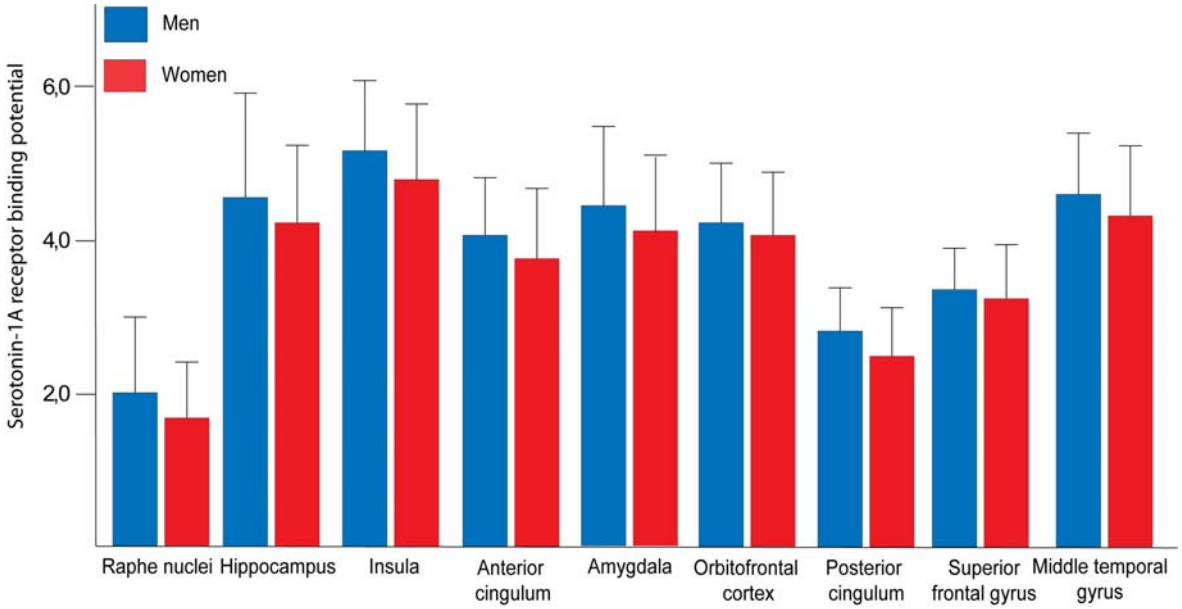

Figure 4.2 Mean 5-HT_{1A} receptor BP_{ND} in men and women using the automated delineation approach. For comparison with the manual approach, nine selected regions of interest are shown. (blue bars - men, red bars – women, error bars – standard deviation).

4.3.3. Overall sex differences in the 5-HT_{1A} receptor BP_{ND} using the automated method

An independent samples two-way ANOVA was done to compare the 5-HT_{1A} receptor BP_{ND} over all 45 regions of interest of the automated method. Radiochemical variables and age were first included as covariates, but as they had no effect on the model (p>.05) they were dropped from further analysis. Results of the ANOVA are given in table 4.9.

Tests of Between-Subjects Effects			
Fix factor	**df**	***F***	**Sig.**
Sex	1, 30	0.80	0.378
Region	43, 1290	128.28	0.000
Sex * Region	43, 1290	0.48	0.993

Table 4.9 Two-way ANOVA investigating the influence of sex on the 5-HT_{1A} receptor BP_{ND} in 45 regions of interest. The ANOVA reveals a significant effect of region but no effect of sex or the interaction term sex by region. df – degrees of freedom

To summarize, the mean 5-HT_{1A} receptor BP_{ND} over all regions of interest was 3.54 ± 0.05 (mean ± SE) for men and 3.32 ± 0.05 (mean ± SE) for women. The two-way ANOVA did not reveal any significant effect for sex ($F_{1,30}$= 0.8, p=.378) and there was no significant interaction between sex and region. *Post hoc* independent samples *t*-tests done in 45 AAL regions of interest did not reveal any significant sex differences. Therefore, the results of the automated method do not support the hypothesis of sex differences in the 5-HT_{1A} receptor BP_{ND} or in the 5-HT_{1A} receptor distribution.

4.4. SUMMARY

Both the manual and the automated method revealed no significant sex differences in the 5-HT_{1A} receptor BP_{ND} except for a trend in the hypothalamus (p=.012) that did not withstand the Bonferroni correction. Female and male participants did not differ significantly by age, radiochemical variables, regional tracer delivery (R_1) or binding in the reference region (p>.05). There was no effect of age or radiochemical variables on the 5-HT_{1A} receptor BP_{ND} when using them as covariates in the ANOVA (p>.05).

5. DISCUSSION

The main finding of this *in vivo* study is the lack of a sex-specific 5-HT_{1A} receptor binding in 32 healthy male and female subjects. This *in vivo* investigation using PET and the highly specific radioligand [*carbonyl*-^{11}C]WAY-100635 did not confirm the hypothesis of sex differences in the 5-HT_{1A} receptor BP_{ND} in any brain region suggested in some previous PET studies (Parsey et al. 2002; Jovanovic et al. 2007). Our results were obtained independently using two delineation methods for regions of interest – a manual delineation and an automated delineation using a PET template and the non-invasive SRTM (Gunn et al. 1998).

5.1. METHODOLOGICAL CONSIDERATIONS

5.1.1. Comparison with previous studies

The absence of sex effects in the 5-HT_{1A} receptor BP_{ND} is in line with several human post mortem (Matsubara et al. 1991; Dillon et al. 1991; Palego et al. 1997) and *in vivo* studies (Meltzer et al. 2001; Moller et al. 2007). Interestingly, the mean 5-HT_{1A} receptor BP_{ND} tended to be lower in females in all regions of interest, which is in contrast to three previous studies using the same radioligand in healthy subjects (Parsey et al. 2002; Jovanovic et al. 2007; Bhagwagar et al. 2003).

One of these studies found a higher 5-HT_{1A} receptor BP_{ND} in females reporting a clearly broader age range compared to our study. The sex difference was found only when using arterial input function but not when using the non-invasive SRTM (Parsey et al. 2002). A second, subsequent study also found a higher 5-HT_{1A} receptor BP_{ND} in females using both arterial input function and the SRTM for comparison (Bhagwagar et al. 2003). Limits of this study were a small sample size (6 females and 8 males) and a broad age range in males (25-65 years) while the age range for females was quite narrow (47-52 years). Both groups did not control for the possible effects of gonadal steroids (Bethea et al. 2002). A third study, however, found a significantly higher 5-HT_{1A} receptor BP_{ND} using SRTM in females

in a demographically comparable sample to ours (Jovanovic et al. 2007). Given the similar study design (control for hormonal status and young age of the participants), it remains unclear why the suggested sex difference in the 5-HT_{1A} receptor binding was not confirmed by the present investigation. Even more surprising, the mean 5-HT_{1A} receptor BP_{ND} in the present study was *lower* (though not significantly) in females compared to males in every region examined which is the opposite of the findings of Jovanovic et al. (Jovanovic et al. 2007).

5.1.2. Limitations of PET studies

Several issues and limitations need to be considered in the interpretation of these results. First, the disaccording results may reflect a small effect size of sex which would conflict with the inherent limitations of PET studies. These include the particularly high inter-subject variability compared to the broad overlapping range between male and female 5-HT_{1A} receptor BP_{ND} (Rabiner et al. 2002) and the small sample size (because of ethical considerations and high costs of PET measurements). Second, given the significant correlations between the 5-HT_{1A} receptor BP_{ND} and personality traits as aggression (Parsey et al. 2002) or anxiety (Tauscher, Bagby et al. 2001), recruiting by advertisement and random inclusion of subjects in the lower or higher range in personality scales might bias the study sample. Methodological bias, however, is unlikely as the present results have been obtained using two independent approaches for definition of brain regions after control for sex differences in binding of the reference region. In addition, two independent approaches for quantification have been applied, the SRTM and the Logan non-invasive approach that yielded comparable results in the ANOVA (data derived from the Logan approach not shown). By using an automated delineation, for the first time a comprehensive distribution map of the 5-HT_{1A} receptor BP_{ND} in 45 regions of interest has been obtained in males and females.

5.1.3. Sex-dependent age effects on the 5-HT_{1A} receptor BP_{ND}

Another contributing factor for diverging results may be the sex specific effect on age-dependent increase or decrease in the 5-HT_{1A} receptor binding. Several post mortem studies using the 5-HT_{1A} receptor agonist [^{3}H]8-OH-DPAT as radioligand did not report

any significant sex differences in receptor binding or distribution while an age-dependent decline of binding sites was found in men but not in women (Dillon et al. 1991; Matsubara et al. 1991; Palego et al. 1997). In women, the 5-HT_{1A} receptor binding in the occipital cortex even seemed to increase with age (Palego et al. 1997). A similar trend was also observed in recent human PET studies using the radioligand [*carbonyl*-^{11}C]WAY-100635. Here, a significant inverse correlation of the 5-HT_{1A} receptor BP_{ND} with age was found in men (Tauscher, Verhoeff et al. 2001; Moller et al. 2007) but not in women (Meltzer et al. 2001). Therefore, comparing the 5-HT_{1A} receptor expression in inhomogeneous groups of subjects with regard to age may yield deceptive results. The 5-HT_{1A} receptor BP_{ND} may be higher in females when comparing men and women in older age but lower in young adulthood. The results of the present study would be in line with this hypothesis, since the trend towards a lower mean 5-HT_{1A} receptor BP_{ND} in females was observed in a particularly young sample of volunteers.

5.1.4. Effects of gonadal steroids

The sex-dependent age effect on the 5-HT_{1A} receptor binding might be furthermore associated with lifetime changes in the production of gonadal steroids in men and women. Evidence is found that serotonin availability might be dependent on steroid hormones as no gender differences in serotonin levels are found when comparing male rats to ovariectomized female rats (Duval and Mignot 1985). Serotonin levels have also been reported to vary with estrous cycle (Kueng et al. 1976; Gundlah et al. 1998) and endocrine state as pregnancy and post partum (Klink et al. 2002) and to be influenced by steroid application (Munaro 1978; Bitar et al. 1991; Di Paolo et al. 1983). Serotonin synthesis has furthermore been shown to increase after castration which can be counteracted by testosterone administration (Engel et al. 1979). In a study with healthy postmenopausal women, a higher 5-HIAA excretion after long-term treatment with estradiol was demonstrated (Lippert et al. 1996)

An abundant amount of literature demonstrates the influence of steroid hormones on the 5-HT_{1A} receptor expression. Long-term administration of estrogen was shown to increase the activity of the tryptophan hydroxylase (Pecins-Thompson et al. 1996) while down regulating the pre- and postsynaptic 5-HT_{1A} receptor binding (Pecins-Thompson and

Bethea 1999; Lu and Bethea 2002). The effect was particularly pronounced in the raphe nuclei and in the hippocampus, i.e. in regions with a lower 5-HT_{1A} receptor binding potential in female rodents when compared to males (Schiller et al. 2006). A phase effect of the menstrual cycle on the 5-HT_{1A} receptor BP_{ND} was demonstrated in preclinical research (Flugge et al. 1999) and indicated in a recent PET study which however lacked statistical power (Jovanovic et al. 2006). Effects of progesterone on the 5-HT_{1A} receptor binding have been reported by our group (Spindelegger, Mitterhauser et al. 2008). Since we observed a slightly lower 5-HT_{1A} receptor BP_{ND} in women measured in the early follicular phase versus women measured in the late follicular phase, this might be indicative of a down regulation of the receptor by high progesterone levels at the end of the menstrual cycle with a recovery of the 5-HT_{1A} receptor BP_{ND} later in the cycle. However, as long as the temporal relation of changes in the 5-HT_{1A} receptor BP_{ND} with regard to steroid hormone plasma levels is not known, these assumptions remain highly speculative.

An increased serotonin neurotransmission caused by the long term down regulation of somatodendritic 5-HT_{1A} receptors by higher estrogen and progesterone levels in females would be in line with animal studies that demonstrated a higher serotonin synthesis and turnover (Haleem et al. 1990) and overall higher serotonin levels (Dickinson and Curzon 1986) in the brain of female rodents. According to this hypothesis, a slightly lower overall 5-HT_{1A} receptor expression in females as indicated in the present study would lead to a reduced serotonergic inhibition on postsynaptic mainly glutamatergic neurons (Fink and Gothert 2007) and increased serotonin turnover (Drossopoulou et al. 2004). This would be compatible with a greater serotonergic responsiveness in females. Indirectly, the higher prevalence rate of mood disorders in women that becomes apparent only with the onset of puberty while diminishing after the menopause might be an evidence for the effect of ovarian steroids on the serotonin neurotransmission (Bebbington 1996). Therefore, the restriction of female participants to women with regular menstrual cycle duration and the conductance of PET measurements in a restricted phase of the menstrual cycle appear as important prerequisites for revealing an either higher or lower 5-HT_{1A} receptor BP_{ND} in women compared to men.

5.1.5. Absence of sex effects on the serotonin-1A receptor

Finally, it must be considered that the hypothesis of sex differences in the human serotonergic system may be simply false. Indeed, the present study is the fourth example of differing results with regard to sex effects on the serotonergic system. The serotonin synthesis has been reported both lower in females (Nishizawa et al. 1997; Sakai et al. 2006) and lower in males (Chugani et al. 1998). The 5-HTT binding potential was reported to be both higher in females (Staley et al. 2001) or higher in males (Jovanovic et al. 2007). In a large study sample of 88 subjects, Praschak-Rieder et al. found no sex differences in 5-HT binding (Praschak-Rieder et al. 2008). Another study with 52 healthy subjects did not reveal any sex differences in the 5-HT_{2A} receptor binding (Adams et al. 2004), which was suggested before by Biver et al. (Biver et al. 1996). Therefore, sex differences in the incidence of affective disorders might be not adequately explained by sex differences in serotonergic receptor or transporter densities.

5.2. CONCLUSION

In summary, our results do not confirm the hypothesis of sex differences in the 5-HT_{1A} receptor BP_{ND} in 16 healthy young women when compared to 16 healthy young men. This was demonstrated using two independent delineation methods (manual vs. normalized ROI template-based) and a non-invasive quantification approach (SRTM). The automated delineation method was shown reliable in comparison with the manual delineation with the additional benefit of a high number of regions of interest and a lower variability in regional volumes. A slightly, though not significantly, lower mean 5-HT_{1A} receptor BP_{ND} in female subjects was observed in all regions investigated which is in contrast with some previous studies. Given the high intersubject variability of the 5-HT_{1A} receptor BP_{ND} within both sexes, and the usually low sample size in PET studies, the inclusion criteria of study samples might significantly influence results on sexual dimorphisms. The higher prevalence rates of depression and anxiety in women, which have been associated with alterations of the 5-HT_{1A} receptor, can not be sufficiently explained by sexual dimorphisms in the 5-HT_{1A} receptor affinity, density or distribution. The biological basis of the female vulnerability to affective disorders remains therefore open for future investigation.

Figures

Tables

Abbreviations

5-HIAA	5-hydroxyindole acetic acid
5-HT	serotonin
5-HT_{1A}	serotonin-1A receptor
5-HT_{2A}	serotonin-2A receptor
5-HTT	serotonin transporter, SERT
AADC	L-aromatic amino-acid decarboxylase
ACTH	adrenocorticotropin releasing hormone
ANOVA	analysis of variance
BMI	body mass index
BP_{ND}	non-displaceable binding potential
CSF	cerebrospinal fluid
GABA	γ-aminobutyric acid
GBq	gigabecquerel
HPLC	high performance liquid chromatography
keV	kilo electron volt
LH	luteinizing hormone
MBq	megabecquerel
MRI	magnetic resonance imaging
PET	positron emission tomography
ROI	region of interest
SRTM	simplified reference tissue model
TAC	time activity curve
TPH	tryptophan hydroxylase

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