Effects of progesterone and estradiol on the human serotonergic neurotransmission

An investigation of 5-HT_{1A} receptor distribution using PET and [carbonyl-^{11}C]-WAY-100635

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… gewidmet meinem Vater
1946-1999

SUMMARY

Background: The serotonergic neurotransmission plays an important role in the pathophysiology of affective states. The serotonin-1A receptor (5-HT_{1A}) regulates the serotonergic firing rate in the raphe nuclei and the serotonergic inhibition an GABAergic and glutaminergic neurons. Reduced 5-HT_{1A} levels have been shown in patients suffering from psychiatric disorders including depression and anxiety disorders. Moreover, gender differences in mood and affective disorders have been frequently reported. This may indicate a strong influence of sex steroid hormones on affective and mood disorders. Findings in several animal studies in rodents and non-human primates have shown that estradiol and progesterone can modulate the serotonergic system by genomic mechanisms. The expression of serotonin receptors such as 5-HT_{1A} and 5-HT_{2A} has been reported to be modulated by steroid hormones and thus might play a causal role in the pathomechanism of psychiatric disorders.
Aim: The aim of this study was to investigate the relationship between the steroid hormones progesterone and estradiol plasma levels and the 5-HT_{1A} receptor distribution in humans using PET and the 5-HT_{1A} specific ligand [carbonyl-^{11}C]-WAY-100635.
Methods: 18 healthy male volunteers (28.17 ± 8.33 years, mean age ± SD) were examined by a GE Advance PET scanner and [carbonyl-^{11}C]-WAY-100635. Dynamic scans (in 3D mode) started simultaneously with a bolus injection using an average dose of 5.66 ± 0.85 MBq/kg of body weight. A series of 30 time frames (15x1min, 15x5min) resulted in a total acquisition time of 90 minutes. T1-weighted MR images have been co-registered to PET_{ADD} images applying SPM2. Nine regions-of-interest (ROI; cerebellum, anterior cingulate cortex, insula, orbitofrontal cortex, amygdala, hippocampus, motor cortex, visual cortex, dorsal raphe nucleus) were delineated on the co-registered anatomical images using PMOD 2.55 and anatomical criteria established by Bremner et al. Quantification of 5-HT_{1A} receptor binding potential was performed with PMOD and the Simplified Reference Tissue Model (SRTM) based on a two-tissue compartment model.
Results: This investigation revealed a significant negative correlation between progesterone plasma levels and the presynaptic 5-HT_{1A} autoreceptors binding potential in the dorsal raphe nuclei. Furthermore, a highly significant negative correlation between the progesterone plasma level and postsynaptic 5-HT_{1A} receptor binding potential in the amygdala was found. A multiple regression analysis including the 5-HT_{1A} as dependent variable and progesterone plasma levels and age as independent variables showed significant results in the hippocampus, insula, amygdala, orbitofrontal cortex, motor cortex as well as in the dorsal raphe nuclei. Estradiol as an additional independent variable improved the significance levels in the anterior cingulate cortex, the hippocampus and the amygdala region.
Conclusion: Comparing these results to steroid hormone-induced increase of excitatory 5-HT_{2A} receptor density on glutaminergic and GABAergic neurons, these data suggest a shift of the balance between the serotonergic inhibitory and excitatory effects on these neurons mediated by steroid hormones. Therefore, these findings could provide a biological rationale to explain gender differences in affective disorders like depression and anxiety linked to changes of the serotonergic system. An augmentation approach with steroid hormones or steroid derivates in the therapy of anxiety disorders and depression might improve the clinical outcome.

Zusammenfassung

Hintergrund: Das serotonerge System spielt eine wichtige Rolle in der Pathophysiologie affektiver Erkrankungen. Der Serotonin-1A Rezeptor (5-HT_{1A}) regelt die Aktivität serotonerger Neuronen in den Raphekernen und die serotonerge Inhibition an GABAergen und glutaminergen Neuronen. In Patienten, die an Depression oder Angststörungen leiden, wurden verminderte Serotonin-1A Rezeptordichten nachgewiesen, weiters findet man geschlechtsspezifische Unterschiede in der Erkrankungshäufigkeit. Dies legt den Schluß nahe, dass Steroidhormone über serotonerge Mechanismen die Ausbildung affektiver Erkrankungen beeinflussen. In Ratten- als auch in Primatenstudien wurde ein genetischer Mechanismus nachgewiesen, der einen direkten Zusammenhang zwischen Steroidhormonen wie Progesteron und Estradiol und der Expression der 5-HT_{1A} und 5-HT_{2A} Rezeptoren darstellt.
Zielsetzung: Das Ziel dieser Studie war es, einen Zusammenhang zwischen den Progesteron und Estradiol Plasmawerten und der 5-HT_{1A} Rezeptorverteilung im menschlichen Gehirn aufzuzeigen.
Methoden: Achtzehn gesunde männliche Probanden (Alter 28,17 ± 8,33 Jahre) wurden in diese Studie aufgenommen und mittels PET unter der Verwendung des spezifischen Liganden [carbonyl-^{11}C]-WAY-100635 untersucht. Die dynamische Messungen (im 3D Modus) wurden simultan zur Injektion von [carbonyl-^{11}C]-WAY-100635 gestartet. Es wurde eine durchschnittliche Dosis des Tracers von 5,66 ± 0,85 MBq pro Kilogramm Körpergewicht verabreicht. Die Messdauer betrug 90 Minuten, wobei 30 Aufnahmen gemacht wurden (15 Aufnahmen zu je 1 Minute, 15 Aufnahmen zu je 5 Minuten). Zusätzlich wurden bei jedem Probanden eine T1-gewichtete MR Aufnahmen gemacht, die auf das Summationsbild der individuellen PET Aufnahme koregistriert wurde. Auf diesen koregistrierten MR Bildern wurden neun unterschiedliche Zielregionen (das Kleinhirn, das vordere Zingulum, die Insel, der orbitofrontale Kortex, die Amygdala, der Hippokampus sowie der Motorkortex und der Visokortex) nach zuvor definierten Kriterien eingezeichnet. Die Quantifizierung der 5-HT_{1A} Rezeptoren wurde mithilfe eines Zwei-Kompartment-Modells, dem „Simplified Reference Tissue Model“, vorgenommen. Zum Einzeichnen der Zielregionen und der Quantifizierung der 5-HT_{1A} Rezeptoren wurde das Programm PMOD verwendet.
Ergebnisse: Diese Untersuchung zeigte eine signifikante negative Korrelation zwischen den Progesteron Plasmawerten und den prä-synaptischen 5-HT_{1A} Autorezeptoren im dorsalen Raphekern. Weiters wurde eine hoch signifikante negative Korrelation zwischen den Progesteron Plasmawerten und den post-synaptischen 5-HT_{1A} Rezeptoren der Amygdala gefunden. Eine multiple Regressionsanalyse mit der 5-HT_{1A} Rezeptorverteilung als abhängige Variable und den Progesteron Plasmawerten sowie dem Alter als unabhängige Variable zeigte signifikante Ergebnisse im Hippokampus, der Insel, der Amygdala, dem orbitofrontalen Kortex sowie im dorsalen Raphekern. Durch Hinzufügen der Estradiol Plasmawerte als zusätzliche unabhängige Variable verbesserten sich die Signifikanzwerte in dem Regressionsmodel im vorderen Zingulum, dem Hippokampus und der Amygdala.
Konklusion: Frühere Studien haben gezeigt, dass es durch Gabe von Steroidhormon zu einem Anstieg der Dichte der exzitatorisch wirkenden 5-HT_{2A} Rezeptoren auf glutaminergen und GABAergen Neuronen kommt. Diese Ergebnisse gemeinsam mit den Resultaten der vorliegenden Studie können als eine Verschiebung des Gleichgewichts zwischen der inhibitatorischen und exzitatorischen serotogenen Wirkung auf diese Neuronen durch Steroidhormone interpretiert werden. Dieser Mechanismus könnte somit einen biologischen Erklärungsansatz für geschlechtspezifische Unterschiede bei affektiven Erkrankungen im Bezug auf das serotonerge System bieten. Der Einsatz von Steroiden und Steroidderivaten als Augmentationstherapie bei Angsterkrankungen und Depressionen verbessert möglicherweise das klinischen Ergebnis.

CONTENT

1. INTRODUCTION

The serotonergic system plays a pivotal role in the regulation of various physiological functions (Struder and Weicker 2001). Respiration, sleep, cardiovascular activity, temperature, appetite and emesis are regulated or modulated by serotonergic neurons in the brainstem (Azmitia 1999). Moreover, the serotonergic system is implicated in the regulation of affective states including anxiety, fear and aggression (Baldwin and Rudge 1995) as well as memory and cognition (Buhot et al. 2000). The serotonergic neurotransmission is regulated by at least fourteen different receptors. Reduced serotonin-1A (5-HT_{1A}) receptor levels have been shown in patients suffering from psychiatric disorders including depression (Drevets et al. 1999; Meltzer et al. 2004; Owens and Nemeroff 1998; Stockmeier 2003), anxiety disorders (Kasper 2001; Neumeister et al. 2004) and schizophrenia (Kasper et al. 1999; Tauscher et al. 2002). There is substantial evidence that an imbalance of the serotonergic system is involved in the pathophysiology of the psychiatric disorders mentioned before (Asberg et al. 1976; Lesch 2005; Lopez-Figueroa et al. 2004; Van Praag 1982).

Gender differences in mood and affective disorders such as depression and anxiety have been reported frequently (Angst et al. 2002; Breslau et al. 1995; Kessler et al. 1993; Kessler et al. 1994; Robins et al. 1984). For example, the prevalence for depression during puberty in girls is twice the rate than in boys (Angold and Worthman 1993; Silberg et al. 1999). This may indicate a strong influence of sex steroid hormones on affective and mood disorders (Fink et al. 1998). Findings in several animal studies in rodents and non-human primates support the assumption that estradiol and progesterone can modulate the serotonergic system by genomic mechanisms. The expression of the serotonin transporter (SERT) and several serotonin receptors such as 5-HT_{1A} and 5-HT_{2A} has been reported to be modulated by steroid hormones and thus might play a causal role in the pathomechanism of neuropsychiatric disorders (Bethea et

al. 2000; Bethea et al. 2002; Bethea et al. 1998). Recent technical advances enable the *in vivo* visualization of serotonergic receptors by means of positron emission tomography (PET).

At the beginning, this thesis summarizes the hypotheses tested, followed by a short overview of the serotonergic system and the steroid hormones progesterone and estradiol. The methodical part of this paper then describes the details concerning methods and data analysis approaches applied in this investigation. The results chapter summarizes the findings which are interpreted in a final discussion including an outlook and suggestions for further investigations in this field.

2. AIM

The aim of this study was to investigate the relationship between the steroid hormones progesterone and estradiol and the 5-HT_{1A} receptor distribution. As demonstrated in rodents and non-human primates, steroid hormones cause a change in the 5-HT_{1A} receptor density in the dorsal raphe nuclei and cortical as well as subcortical areas expressing steroid receptors. For the first time, this study intends to show a correlation between progesterone and estradiol plasma levels and the 5-HT_{1A} receptor levels in humans.

3. HYPOTHESES

In order to investigate the interaction of the steroid hormones progesterone and estradiol with the serotonergic system the following hypotheses were tested:

1. Progesterone and estradiol plasma levels correlate with the presynaptic 5-HT_{1A} autoreceptor binding potential in dorsal raphe nucleus.

2. Progesterone and estradiol plasma levels correlate with the postsynaptic 5-HT_{1A} receptor binding potential in several cortical and subcortical areas.

3. The influence of progesterone and estradiol is significantly pronounced in brain regions expressing high levels of steroid hormone receptors.

4. BACKGROUND

4.1. The serotonergic system

The monoamine serotonin was initially identified in 1948 (Rapport et al. 1948). Twarog and Page discovered the existence of serotonin in the mammalian brain in 1953 (Twarog and Page 1953) and Marrazzi and Hart revealed the properties of serotonin as a neurotransmitter (Marrazzi and Hart 1955). Only 1-2% of the serotonin in the body can be found in the brain, whereas the remainder is located in platelets, mast cells and enterochromaffine cells of the gastrointestinal tract (Gershon et al. 1985).

4.1.1. Structure and pathways of the serotonergic system in the human brain

In 1911, Ramón y Cajal described the special characteristics of giant cells in the brainstem midline today known as the somata of serotonergic neurons (Ramon y Cajal 1911). By using histochemical fluorescence techniques, Dahlstrom and Fuxe identified nine different groups of serotonergic neurons (B_1 – B_9) extending form the midbrain to the cervical spinal cord in the 1960s (Dahlstrom and Fuxe 1964). Later, Azmitia et al. delineated main projection pathways, five ascending and three descending from serotonergic brainstem neurons (Azmitia and Gannon 1986; Azmitia and Segal 1978). Wallace and Lauder differentiated two groups of serotonergic neurons: A superior group between midbrain and pons (according to Dahlstrom and Fuxe B5-B8), and an inferior group ranging from the caudal pons to the cervical spinal cord (B1-B4) (Wallace and Lauder 1983).

The superior part consists of two groups of 5-HT neurons, namely rostral and caudal. The former builds the main body of the dorsal raphe nucleus and the caudal linear nucleus (B6, B7), the latter forms the median raphe nucleus and the interfascicular

portion of the dorsal raphe nucleus (B5, B8) (Figure 1). Given the fact that the neurons of dorsal raphe nuclei are the largest group of serotonergic neurons projecting to cortical and subcortical areas, the next chapter the focuses on this region.

The dorsal raphe nucleus (DRN) consists of a medial, a lateral and a caudal portion, whereas the medial comprises a mediodorsal (superior) and an interfascicular part (Azmitia and Whitaker-Azmitia 2004). The neurons of the interfascicular portion mingle with the caudal median raphe nucleus (MRN). The majority of the DRN is accounted for by the lateral component which can be categorized in a dorsal and ventral part (Tork 1990).

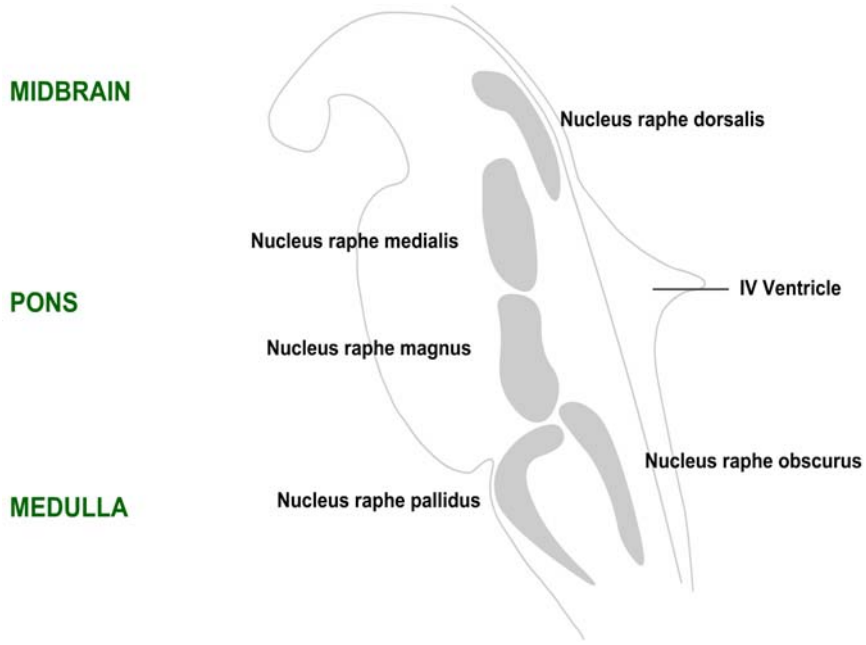

Figure 1. **The raphe nuclei**
Differentiation of the raphe nuclei in the human brainstem

The five pathways projecting to the forebrain mainly originate from the superior raphe nuclei (Azmitia and Segal 1978) and three project to the spinal cord (Alvarez et al. 1998)

(Figure 2). The serotonergic fibers vary according to their diameter, myelination and interaction via synaptic or non-synaptic connections (Azmitia and Segal 1978; Hornung et al. 1990).

Two main serotonergic pathways initiating from different starting points in the DRN and the MRN finally join, mix and split again at the caudal diencephalons. These serotonergic fibers project on the one hand to the lateral cerebral cortex, on the other hand to the basal forebrain, the amygdala, hypothalamus, the cingulum and finally terminate in the medial cerebral cortex and the hippocampal region (Hornung 2003).

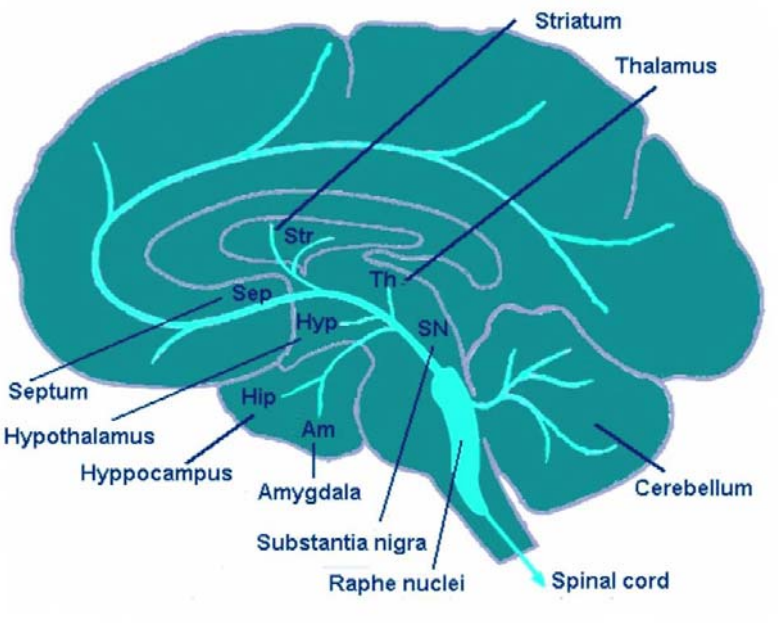

Figure 2. **Serotonergic pathways**
The main serotonergic pathways projecting form the raphe nuclei to cortical and subcortical regions.

4.1.2. Synthesis and metabolism of Serotonin

The chemical identification of serotonin goes back to Rapport (Rapport et al. 1948), who examined the structure of this indoleamine evolving from the essential aminoacid tryptophan, which can be found in high concentration in serotonergic neurons. There, this aminoacid is hydroxylated by tryptophanhydroxylase resulting in 5-hydroxytryptophan, which is decaboxylated by 5-hydroxytryptophandecarboxylase to serotonin (figure 3). The enzyme tryptophanhydroxylase which is located in serotonergic neurons acts as pacemaker of the serotonin synthesis and is commonly not saturated (Ganong 1999). This implies that the velocity of the synthesis can be modified for example by glucocorticoids which increase or parachlorphenalamin which decreases the reaction rate (Hamon et al. 1978). Furthermore, a change of the intake of tryptophan causes a change of the cerebral serotonin synthesis (Neumeister et al. 1997; Neumeister et al. 1999). Serotonin is stored in vesicles of neurons and can be released into the synaptic cleft. There are also serotonergic neurons which do not build common synapses but release serotonin to brain tissue and the ventricle system by means of non-synaptic interactions (Azmitia and Segal 1978; Hornung 2003).

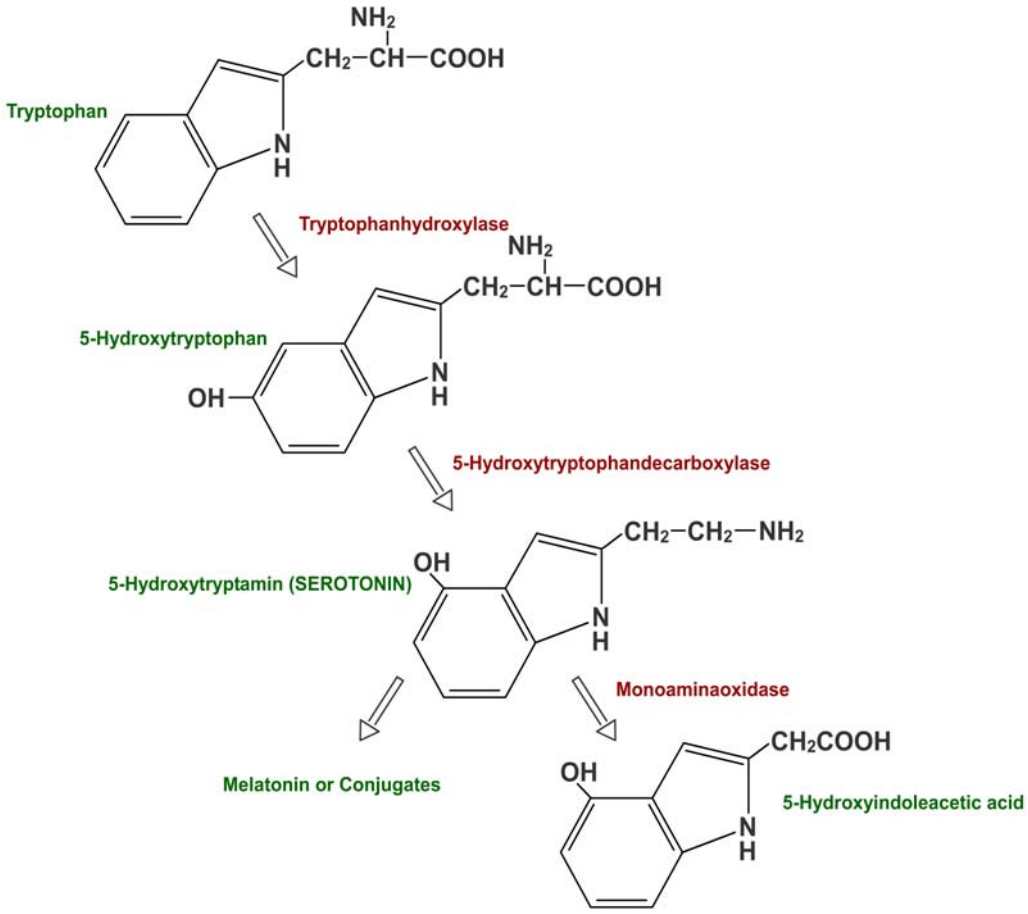

Figure 3. **Biosynthesis of the monoamine serotonin**
Different steps of biosynthesis of serotonin with the respective enzymes (Ganong 1999).

The serotonin in the synaptic cleft (figure 4) can either be reuptaken into the presynaptic neuron by an Na^+/K^+-ATPase dependent SERT or inactivated by the extracellular enzyme monoaminooxidase type A (MAO_A) into 5-hydroxyindoleacetic acid (5-HIAA) (Ganong 1999). Serotonin reuptaken by SERT can also be inactivated to 5-HIAA by an intracellular monoaminooxidase type B (MAO_B) or recaptured in the storage vesicles for reutilisation. The 5-HIAA is the main urinary metabolite of serotonin thus indicating the amount of serotonin in the human body.

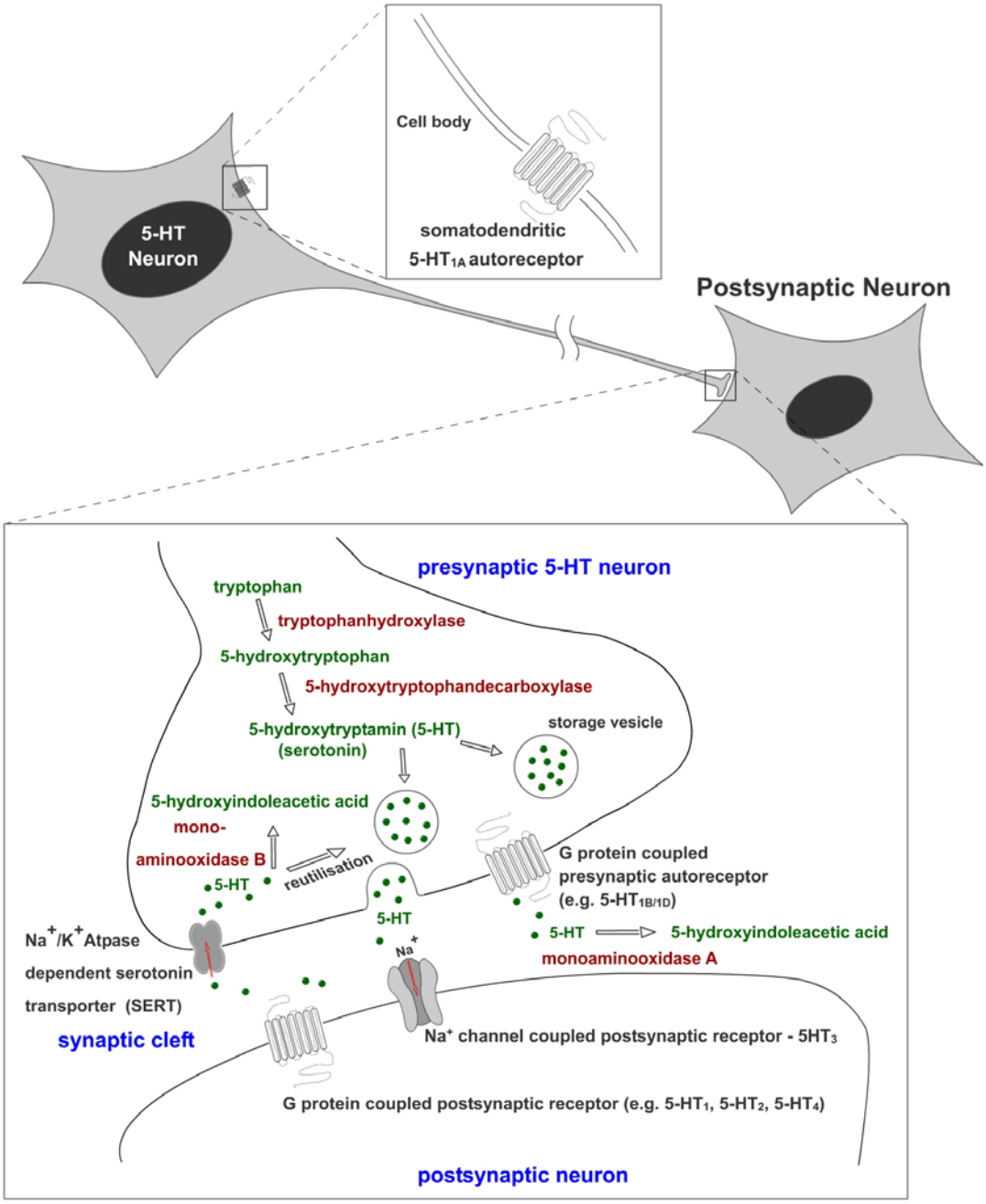

Figure 4. **Synaptic cleft of serotonergic neurons**

Serotonergic neurotransmission and serotonin release into the synaptic cleft as well as reuptake and utilisation of serotonin.

(Adapted and modified from Kent et al. 2002).

4.1.3. Serotonin receptors

The serotonergic system and changes of 5-HT receptors and transporters have been linked to the pathogenesis of psychiatric disorders as schizophrenia (Kasper et al. 1999; Meltzer et al. 2003; Tauscher et al. 2002; Tauscher et al. 1999), depression (Drevets et al. 1999; Meltzer et al. 2004; Owens and Nemeroff 1998; Stockmeier 2003) and anxiety disorders (Neumeister et al. 2004; Tauscher et al. 2001a). Due to their heterogeneity the serotonin receptors have been classified in seven different families (5-HT_1-5-HT_7), all of which except the 5-HT_3 are G-protein coupled receptors affecting adenylyl cyclase or phospholipase. The 5-HT_3 as well as the nicotinic cholinergic receptors, is a ligand-gated ion channel (Ganong 1999). The focus of this paper lies on the influence of hormones on the 5-HT_{1A} receptors in the human brain.

4.1.3.1. The 5-HT_{1A} receptor

5-HT_1 receptors can be divided into five subtypes, namely 5-HT_{1A} ,5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F} which all are adenylyl cyclase inhibitors. Because of its similarity (it is positively coupled to phospholipase C) to the 5-HT_2 group the missing 5-HT_{1C} ranks among this group. The G-protein-coupled 5-HT_{1A} receptor (structure shown in figure 5) is located in the lipid bilayer and consists of seven membrane-spanning domains equipped with intracellular and extracellular domains (Shih et al. 1991).

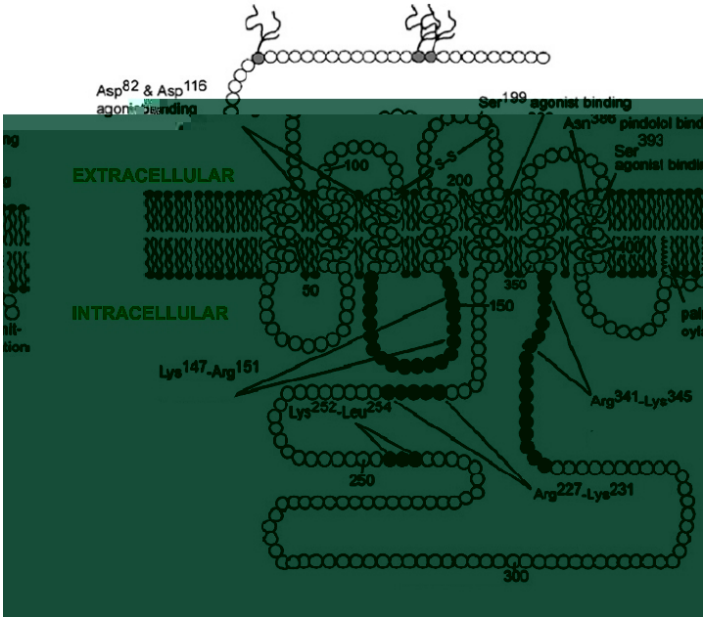

Figure 5. **Structure of the 5-HT$_{1A}$ receptor**

The figure depicts the human 5-HT$_{1A}$ receptor embedded in the lipid bilayer cell membrane. Seven transmembrane α-helices form part of the receptor sequence. Circles represent the amino acids of the receptor sequence.

(Adapted and modified from Pucadyil et al. 2005).

The 5-HT$_{1A}$ receptor leads via voltage-sensitive K^+ channels to a hyperpolarisation of the neuron. As published in several animal and human post-mortem studies (Hall et al. 1997; Varnas et al. 2004), these receptors are located postsynaptically mainly on glutaminergic and GABAergic neurons in cortical areas and the limbic system. In the raphe nuclei, 5-HT$_{1A}$ receptors are located on serotonergic neurons and act as presynaptic somatodentritic autoreceptors lowering the serotonin firing rate when activated (Hamon et al. 1990). The 5-HT$_{1A}$ receptor densities in several cortical and subcortical areas are given in table 3.

4.2. The steroid hormones progesterone and estradiol

The steroid hormones progesterone and estradiol were isolated and characterised in the 1920s (Rubinow et al. 2002) and are not only involved in human sexual maturation and differentiation but also in the regulations of bone maintenance (Turner et al. 1994), mood, memory and cognition (Bethea et al. 2002; Levine et al. 2001). Cholesterol constitutes the main precursor of estradiol and progesterone. Their biosynthetic pathways are depicted in figure 6. Progesterone and estradiol effects can either be mediated by genetic mechanisms via binding to intranuclear steroid receptors or by non-genomic mechanisms as neurosteroids modulating neurotransmitter receptors (Schmidt et al. 2000). Bethea et al. reported the effects of progesterone and estradiol mediated through their specific steroid receptors (progesterone receptor PR; estradiol receptor ER) on the 5-HT_{1A} receptor distribution in non human primates. Based on these findings, our study focuses predominantly on the genomic actions of progesterone and estradiol.

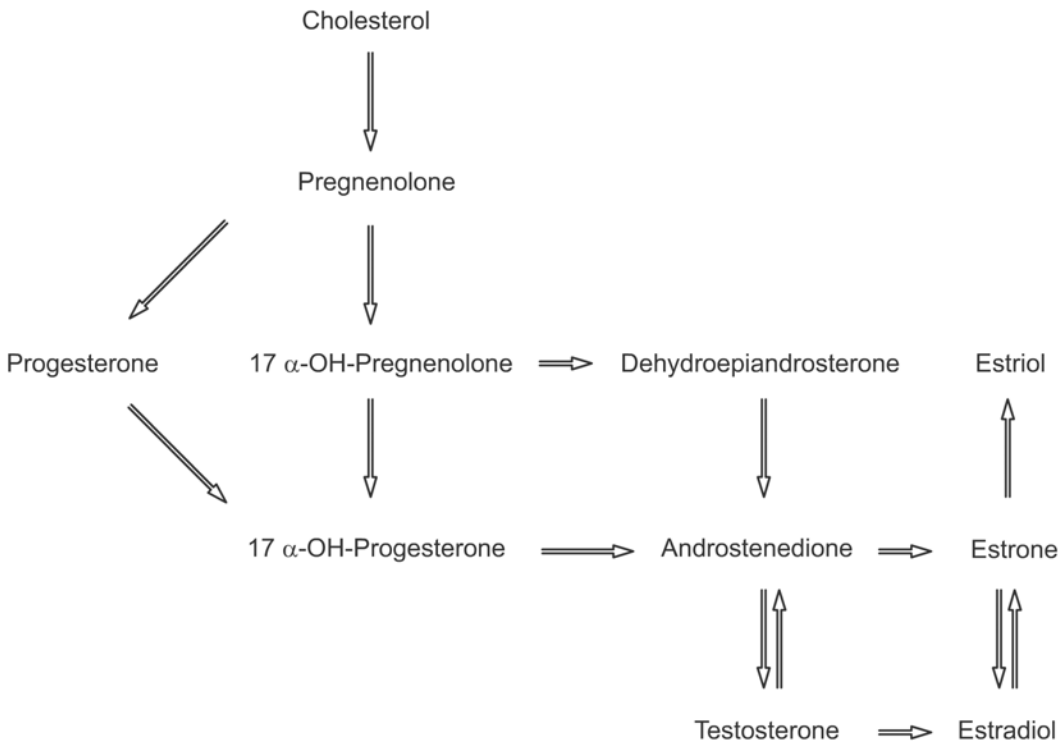

Figure 6. **Biosynthetic pathways of the steroid hormones estradiol and progesterone**
Overview of the biosynthetic pathways leading from the precursor cholesterol to the steroids progesterone and estradiol in the human body. (Adapted from Osterlund and Hurd 2001).

4.2.1. Progesterone

The human steroid progesterone (chemical structure shown in figure 7) plays a key role in the female reproductive system, where it is synthesized cyclically by the ovaries and mediates growth, maintenance and differentiation of the female reproductive tissues (Neumann 1978). Concerning its neuroendocrine functions, progesterone is reported to influence sexual behaviour (Witt et al. 1994). Genomically, the effects of progesterone are mediated through progersterone receptors type A (PR_A) and type B (PR_B) as well as glucocorticoid receptors type I and type II. Additionally, progesterone can be metabolized by the 5α-reductase enzyme which is located in several brain regions into 5α-Dihydroprogesterone. This metabolic product is implicated in the alteration of mood,

fatigue as well as in mechanisms of learning and memory (Celotti et al. 1992; Lephart et al. 1996; Martini et al. 1993).

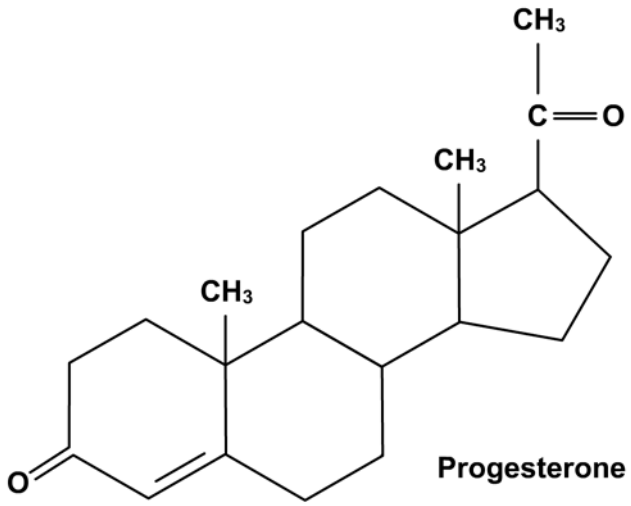

Figure 7. **Chemical structure of the steroid hormone progesterone**

4.2.2. Progesterone receptors

Progesterone receptors exist in two distinct protein isoforms, PR_A and PR_B both arising from the same gene by alternate transcription from two promoters (Giangrande and McDonnell 1999). After diffusion of progesterone through the double layer neuronal cell membrane, the hormone binds to the ligand binding domain of the PR. Thus, the receptor's DNA binding domain is uncapped and binds to the promoter regions of the target gene in order to regulate transcription of the mRNA which is translated into proteins (Gronemeyer 1991).

In the brain the highest expression of PR was found in the hypothalamic ventromedial nucleus and the arcuate nucleus. In limbic areas these receptors were primarily detected in the cingulate cortex, the hippocampus and the medial amygdala (Levine et al. 2001).

The expression of PR is modulated by estrogen in several brain areas via estrogen receptor type α (ERα) (Parsons et al. 1980; Romano et al. 1989). The raphe nuclei show a high density of PRs. There the expression is mediated via estrogen receptor type β (ERβ) (Bethea 1994).

4.2.3. Estradiol

The human estrogens can be subdivided into estrone, estriol and estradiol (chemical structure shown in figure 8), whereas the latter constitutes the most potent natural estrogen (Osterlund and Hurd 2001). In men, 15-20% of the synthesized estradiol are accounted for by the testes, 60% from peripheral aromatisation and 20% peripheral conversion of estrone (Baird et al. 1969). About 2-3% of estradiol circulating in the human blood system are free, whereas the remainder is bound mainly to serum albumin (60%) and to sex hormone binding globulin (SHBG) (Dunn et al. 1981). Only the fraction of estradiol which is not bound to SHBG shows bioactivity (de Ronde et al. 2003). Because of its lipophilic behaviour, estradiol can diffuse through the cell membrane without a specialised transport protein. Furthermore, this steroid hormone is supposed to use a lipid mediated transporter in order to pass the blood brain barrier (Poisson et al. 1984).

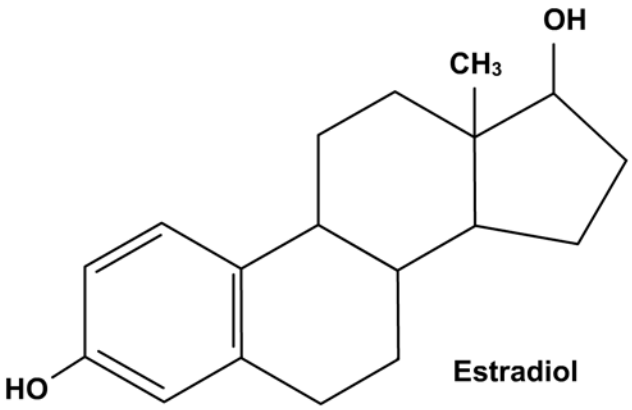

Figure 8. **Chemical structure of the steroid hormone estradiol**

In several brain areas such as the hypothalamus or the limbic system the enzyme aromatase P450 converts androgens into estrogens (Horvath et al. 1997). This locally synthesized estradiol has been reported to be essentially involved in non-genomic actions on neurotransmitter receptor expression (Lephart et al. 2001). Recent studies provide evidence of estradiol to be involved in the regulation of mood, memory and cognition (McEwen and Alves 1999; Osterlund and Hurd 2001).

4.2.4. Estrogen receptors

An intracellular estrogen receptor was described by Jennsen and Jacobson (Jensen 1962). This so called ERα was supposed to be the only estrogen receptor until Kuiper et al. discovered an additional receptor (ERβ) in rats (Kuiper et al. 1996) and Mosselman et al. identified and characterised this receptor in humans (Mosselman et al. 1996). These receptors, ERα and ERβ, forming part of the steroid receptor superfamily, are located intracellularly and mediate the effects of estradiol by acting as ligand inducible transcription factors like the progesterone receptors mentioned before (Gronemeyer 1991).

Regarding their distribution, ER are manly located in limbic related areas including amygdala, hippocampus formation, the entorhinal cortex, the thalamus and hypothalamus. ERα is predominant in the amygdala and the hypothalamus, whereas ERβ is more frequent in the thalamus, entorhinal cortex and the hippocampus region (Gundlah et al. 2000; Osterlund et al. 2000a). Furthermore, several studies reported a high distribution of ERβ and a lack of ERα in the raphe nuclei (Bethea et al. 1996; Gundlah et al. 2001). In the cerebral cortex only moderate or low levels of both ER subtypes were detected (Osterlund et al. 2000b).

4.3. PET

PET is an imaging method for the *in vivo* detection of administered radio-labelled tracers offering the possibility to visualise human kinetic processes (regional blood flow, glucose or fatty acid metabolism) or quantifications of molecules (receptors, transporters, proteins). Radioisotopes which can be used for the labelling of ligands, constitute the precondition for the visualisation. The most frequently applied radionuclides with their specific half-lives for labelling are shown in table 1. The selection of tracer and radionuclide varies according to the biological processes or receptors examined. In practice, the tracer is injected intravenously, inhaled or ingested.

Radioisotope	**Half-life** [min]
^{11}C	20.4
^{13}N	9.96
^{15}O	2.04
^{18}F	109.8

Table 1. **Isotopes commonly used in PET with their specific half-lives**
(Adapted and modified from Otto and Coenen 2005).

4.3.1. PET Technique

The PET technique requires positron emitting isotopes which are generated by a cyclotron where non-radioactive elements such as C, O, F and N are bombed with protons. A specific tracer is labelled with this positron emitting isotope and will then be injected into the subject. The radioisotope decays in the tissue by emitting a positron which subsequently collides with its antiparticle (the electron) after moving a few millimetres in the annihilation process. Consequently, two identical gamma photons of 511keV emerge moving in nearly opposite directions (180° ± 0,3°) (figure 9).

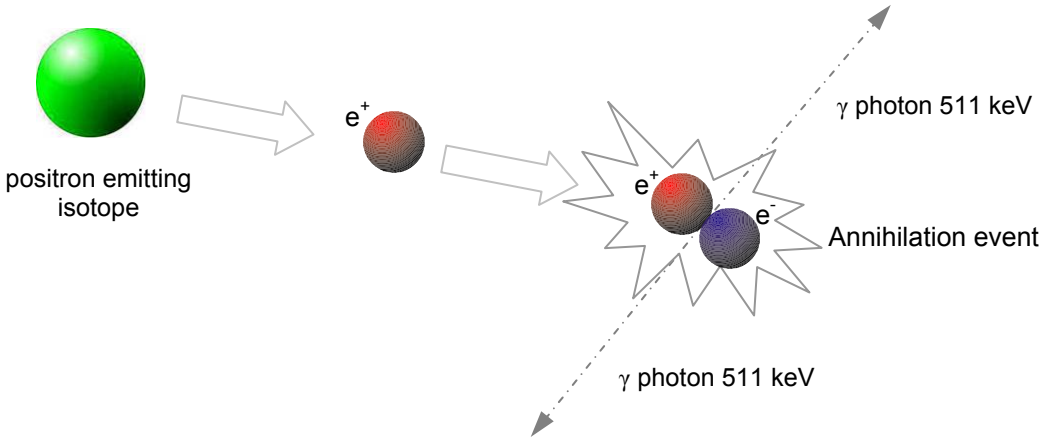

Figure 9. **Positron in annihilation process**

Decay of radioisotope (green sphere) emitting a positron (red sphere), which annihilates with its antiparticle, the electron (blue sphere). After the collision, two gamma photons emerge and depart in opposite directions.

The gamma rays are captured in a so-called coincidence detection which is performed by a PET camera built of one ring equipped with high sensitive scintillation detectors converting the rays into visible light. This process is followed by a final conversion into electric signals using a photomultiplier tube. The detectors located in opposite positions are electronically linked. Only coinciding arrivals at the two connected detectors (occurring within a certain time window of a few nanoseconds) indicate an annihilation process along the line of response (LOR, an imaginary line between these two detectors). In contrary, non-coinciding detections are totally neglected (figure 10).

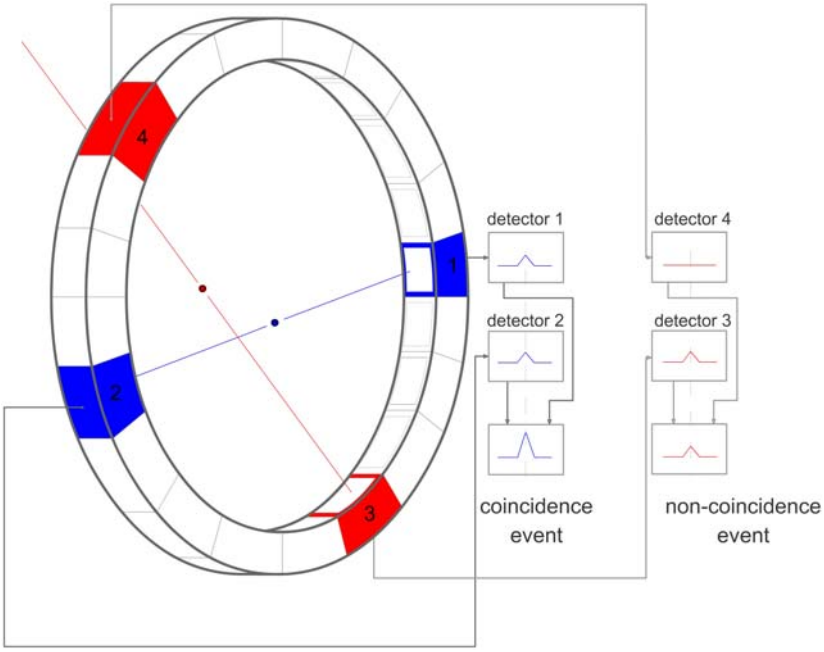

Figure 10. **Coincidence detection of gamma rays in PET (2D mode)**
2D PET ring camera consisting of high sensitive scintillation detectors (1,2,3,4). Coincidence detection of annihilation event occurring on the LOR (blue line) between two opposite detectors (blue 1,2). Non-coinciding and therefore neglected annihilation event (LOR – red line) between two opposite detectors (red,3,4).

When referring to the coincidence events, different types can occur, namely true (as described above), scattered and random ones. Scattered coincidences result from the

Compton scattering event where one photon is deflected from its path. The so-called random coincidence describes a coincident detection of two photons emerging from different annihilation events. Both of these false coincidences imply statistical noise due to the assumption of annihilation events along the wrong LOR (figure 11).

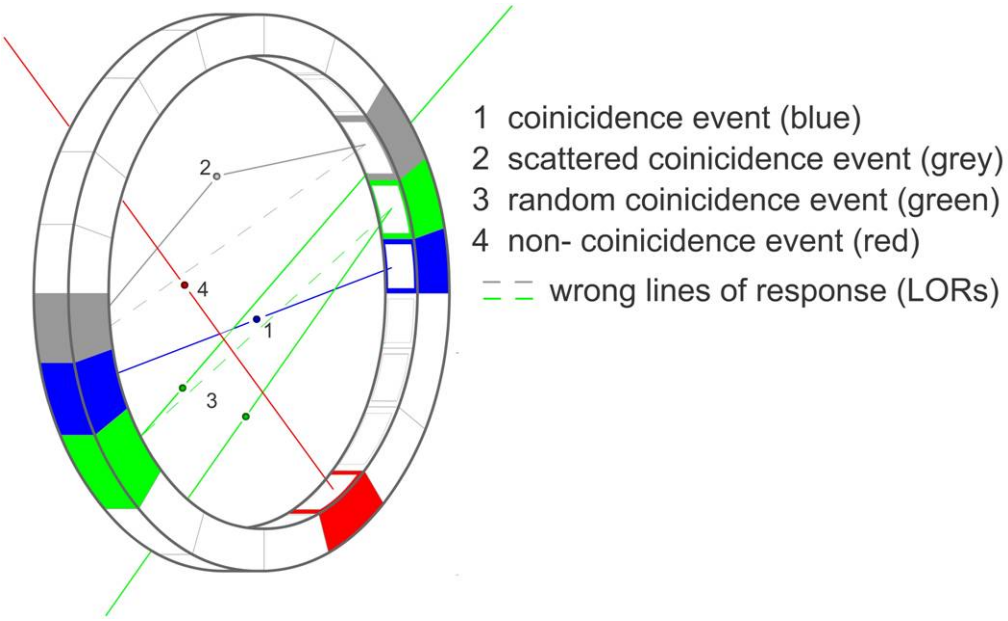

Figure 11. **Types of coincidence detections**

2D PET ring camera consisting of high sensitive scintillation detectors (blue, grey red and green). Normal coincidence detection (blue); scattered coincidence detection (grey); random coincidence detection (green); no coincidence detection (red);

Furthermore, this 2D application can be extended by additional rings (3D) where coinciding detections can occur between opposite detectors located in different rings, thus enabling a higher resolution due to the lower rate of non-coinciding detections (figure 12). The amount and the location (of detector and ring) is computationally

registered and thus provides the possibility to reconstruct images by applying the principles of computed tomography.

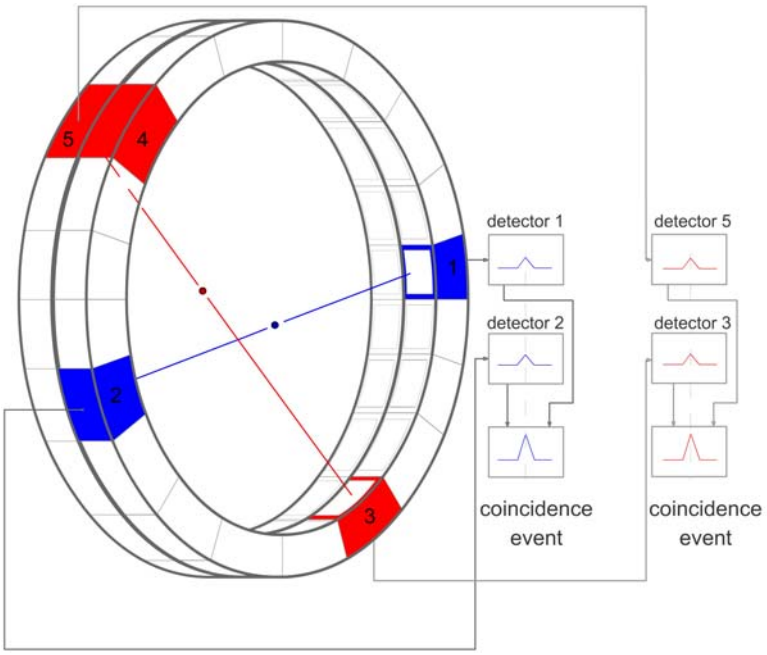

Figure 12. **Principle of a 3D PET camera**

The 3D PET camera consists of at least two rings of high sensitive scintillation detectors (1,2,3,4,5). Coincidence detection of annihilation event can occur on different LORs (blue line, red line) between two opposite detectors of one ring (blue 1,2) or two different rings (red 3,5).

Due to the kinetic energy the positron contains when leaving the nucleus it is able to move over a short distance before colliding with its anti-particle. Consequently, annihilation happens at a distance from the positron's origin. When referring to the isotope ^{11}C labelling the chosen ligand WAY100635, the positron covered a distance of roughly 1mm. The limit of the possible spatial resolution in PET using [carbonyl-^{11}C]-WAY-100635 is physically set over 2 mm.

5. METHODS

The purpose of our study was the investigation of an association between steroid hormone plasma levels and 5-HT_{1A} receptor densities as shown in non-human primates. The regional 5-HT_{1A} receptor densities were investigated using PET and the selective 5-HT_{1A} antagonist ligand [carbonyl-^{11}C]-WAY-100635. Based on the assumption that the steroids progesterone and estradiol play a crucial role in the regulation of the 5-HT_{1A} receptor distribution (Bethea et al. 2002), the peripheral venous plasma levels of both hormones were measured.

5.1. Subjects

This investigation was performed as an non-therapeutic, mono-center study in healthy subjects and approved by the research ethics board of the Medical University of Vienna and the Vienna General Hospital (EK 318/2002 and amendments).
Eighteen healthy men (28.17 ± 8.33 years, mean age ± SD) participated in this study after giving informed consent. The inclusion criteria were:

1. subjects age between 18 and 55 years,
2. no history of psychiatric disorders,
3. no participation in PET or SPECT measurements within 12 months prior to the study,
4. no drug abuse or medication targeting the serotonergic system within one year prior to the study

At the screening visit a physical examination (status, ECG, neurological status) and a routine laboratory screening were done. Furthermore, a psychological exploration was

done including the Spielberger State Trait Anxiety Inventory (STAI) (Balon 2005) and the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al. 1998).
Subjects displaying no clinically relevant abnormalities according to their general physical examination, neurological status, routine laboratory screening or psychological tests were included in the study.

5.2. Data acquisition

In order to measure the 5-HT_{1A} distribution in the human brain *in vivo* PET and the ^{11}C labelled radioligand [carbonyl-^{11}C]-WAY-100635 was used(Gunn et al. 1998; Parsey et al. 2002; Tauscher et al. 2001b). Because of the high specifity and sensitivity of [carbonyl-^{11}C]-WAY-100635, this radioligand can be used for quantification of the 5-HT_{1A} receptor. Due to the fact that the assignment of anatomical structures on PET images is limited, a structural magnetic resonance scan (MR Image) with a 3 Tesla Bruker Biospin MedSpec S300 scanner was done. The calculation of the 5-HT_{1A} receptor binding potential (RBP) was done by indicating the ratio between the maximum concentration of binding sites (B_{max}) and the dissociation constants of the radiotracer (K_D).

5.2.1. PET scanning protocol

The measurements were done by means of a high-spatial-resolution full-ring PET scanner (General Electric Medical Systems, Advance PET Scanner) and the ligand [carbonyl-^{11}C]-WAY-100635. A five minutes transmission scan was performed in 2D mode applying a ^{68}Ge ring source for the correction of tissue attenuation. Dynamic scans (3D mode) started simultaneously with bolus injection of [carbonyl-^{11}C]-WAY-100635 in phosphate-buffered saline (pH 7.4) containing an average activity (mean ± SD) of 5.66 ± 0.85 MBq/kg of body weight. A series of 30 time frames (15x1min, 15x5min) led to a total acquisition time of 90 minutes. The data were scatter corrected and finally

reconstructed applying an iterative algorithm (FORE+ITER). The spatial resolution of the final reconstructed image amounted to full-width half-maximum=4.36mm (FWHM) at the centre of the field (matrix 128x128, 35 slices). No partial volume correction or realignment was done. Figure 13 shows time series of a dynamic PET scan over 90 min acquisition time in a single subject.

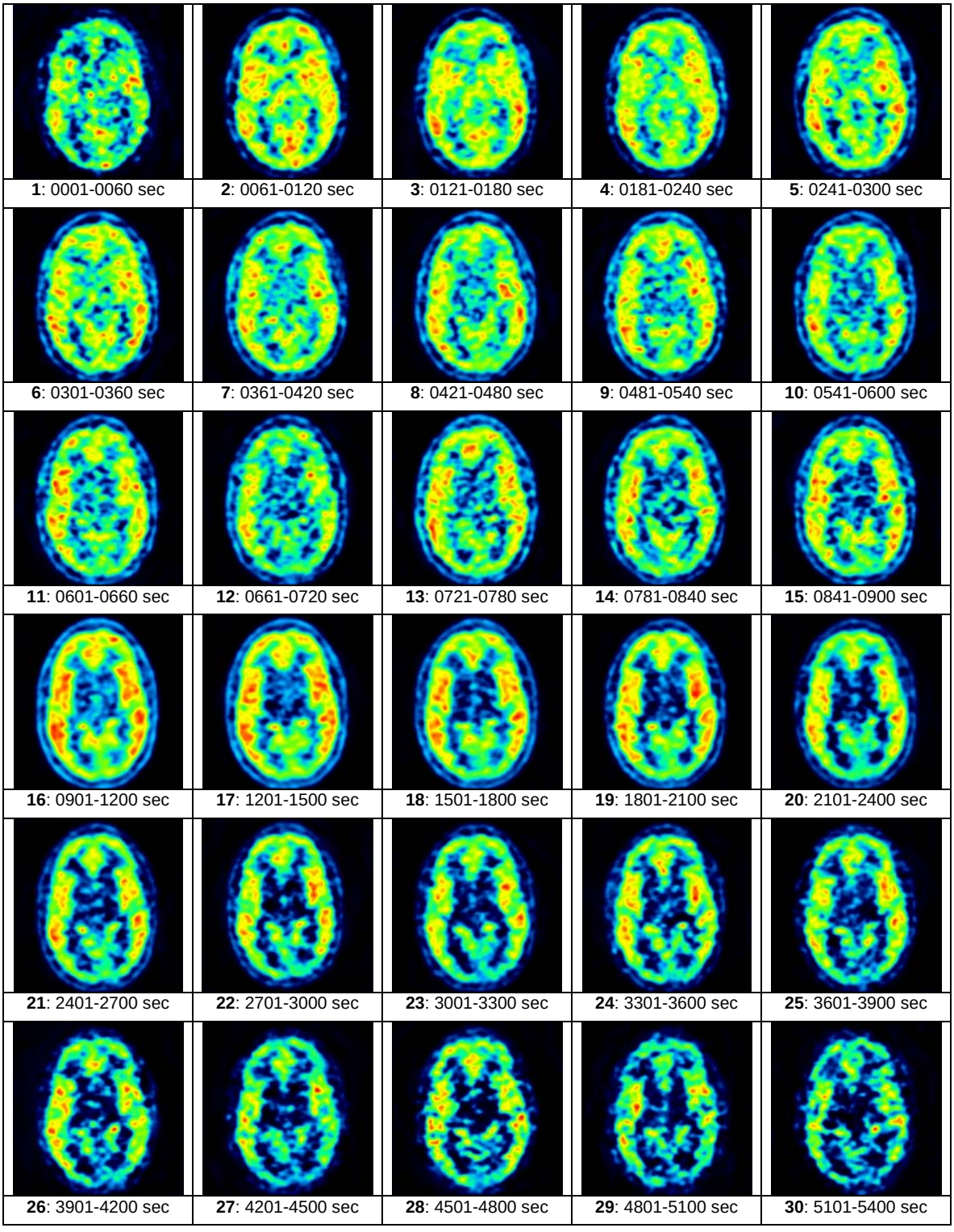

Figure 13. **Dynamic PET scans of 30 sequential time frames in a single subject**

30 time points are depicted. Time frames 1-15 represent an acquisition time of 60 seconds, the last 15 scans (16-30) represent an acquisition time of 300 seconds resulting in a total acquisition time of 90 minutes. The time frames are indicated by bold numbers. The acquisition times are given in seconds.

5.2.1.1. Synthesis of [carbonyl-^{11}C]-WAY-100635

Regarding the delineation of 5-HT_{1A} in animal and human brain the selective antagonist WAY-100635 labelled with carbon-11 is deemed to be a highly effective radioligand (Gunn et al. 1998; Pike et al. 1996; Rabiner et al. 2002). The synthesis of [carbonyl-^{11}C]-WAY-100635 (chemical structure shown in figure 14) for this investigation was carried out with reference to the standard synthetic procedure invented by Pike et al. (Pike et al. 1995; Pike et al. 1996).

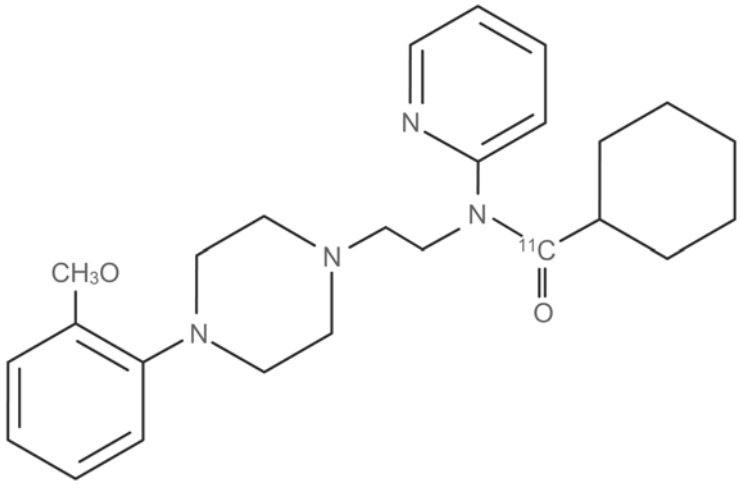

Figure 14. **Chemical structure of the ligand [carbonyl-^{11}C]-WAY-100635**

In practice, [^{11}C]carbon dioxide is sent via a helium stream into a cyclohexylmagnesium chloride in diethylether and tetrahydrofuran (THF) coated polyethene tube. In the next step, a solution consisting of thionyl chloride in THF is passed through this prepared tube in order to convert the former two ingredients into [carbonyl-^{11}C]cyclohexanecarbonyl chloride which is subsequently released into a vial containing 1-(2-methoxyphenyl)-4-(2-(2-pyridylamino)ethyl)piperazine (also known as WAY-100634) plus triethylamine and THF. Finally, [carbonyl-^{11}C]-WAY-100635 can be isolated by means of a preparative high performance liquid chromatography (HPLC) (Matarrese

et al. 2002; McCarron et al. 1996). In addition the determination of the radiochemical identity and purity of [carbonyl-^{11}C]-WAY-100635 is performed by an analytical HPLC which compares retention time of the produced sample with an authentic sample of inactive WAY-100635. The PET center of the Vienna General Hospital routinely evaluates sterility, absence of endotoxines, pH, osmolalilty and residual solvents by standard procedures.

5.2.1.2. Kinetic modelling and Simplified Reference Tissue Model

Tracer kinetic modelling approaches in PET studies are based on a compartmental model (Ichise et al. 2001). For quantification of 5-HT$_{1A}$ RBP the simplified reference tissue model (SRTM) (Lammertsma and Hume 1996) shows better reliability in test-retest studies than approaches based on arterial sampling (Gunn et al. 1998). The SRTM assumes quick exchange rates between the free, non specific and specific binding compartments. Additionally, the distribution volumes of free and non-specific compartments are supposed to be identical in the target and the reference tissue. Furthermore, the free and the non specific compartment at the reference tissue are assumed as one single compartment. The SRTM enables an estimation of the 5-HT$_{1A}$ RBPs (see formula).

$$BP = \frac{B_{max} * f_2}{K_D\left(1+\sum\frac{f_i}{K_i}\right)}$$

In this investigation, the cerebellum was chosen as the reference region because it is less of 5-HT$_{1A}$ receptors (Burnet et al. 1997). The expression B_{max} stands for the

maximum concentration of binding sites, f_2 and f_i are the free fraction of the unbound tracer (f_2) and the competing endogenous ligand (f_i), whereas K_D and K_i represent dissociation constants for the radiotracer (K_D) and for the competing endogenous ligand (K_i) (Rabiner et al. 2002).

5.2.2. Measurement of steroid hormone plasma levels

For the analysis of the steroid hormone plasma levels of progesterone and estradiol venous blood samples were taken twice in the morning hours (between 8:00 and 9:00 a.m.) from the left or right cubital vein. The values of progesterone and estradiol plasma levels were measured by the clinical institute of medical and chemical laboratory diagnostics at the Medical University of Vienna by means of an electrochemiluminescence immunoassay (ECLIA). Due to the fluctuations of the hormone levels, the mean values of the received hormone levels at the two time points were calculated to avoid measurement peaks.

5.3. Data analysis

The quantification of 5-HT_{1A} receptors can be performed by applying an ROI-based or a voxelwise approach. Here, I present the results using the ROI-based approach. Due to the difficulties of a topologically correct delineation of anatomical region on the PET image, the ROIs were drawn on a structural MR image which was co-registered to the subject's PET summation image (PET_{ADD}). The following overview lists the steps of data analysis (table 2).

1. Co-registration of MRI_{T1} to PET_{ADD}
2. Delineation of ROIs on the co-registered MRI_{T1}
3. Generation of TACs for each ROI
4. Calculation of the receptor binding potential applying the simplified reference tissue model by means of decay corrected TACs

Table 2. **Data analysis procedure**
The table shows the four steps for calculation of the 5-HT_{1A} RBP in selected brain regions. MRI_{T1}: T1-weigthed MR image; PET_{ADD}: PET summation image; ROIs: Regions of interest; TACs: Time activity curves

5.3.1. Co-registration of MRI and PET_{ADD}

A PET_{ADD} image (example shown in figure 15b) was generated for each subject as a intensity summation of dynamic PET images of all 30 time points (figure 13) by means of the volume addition tool of PMOD 2.55 (Mikolajczyk et al. 1998). Individual T1-weighted MR images (matrix 256x256x128; individual MR image of one subject see figure 15a) were co-registered to PET_{ADD} image applying SPM2 (Friston et al. 1995). An individual co-registered MR image and a superimposed PET_{ADD} image are depicted in figure 16a,b.

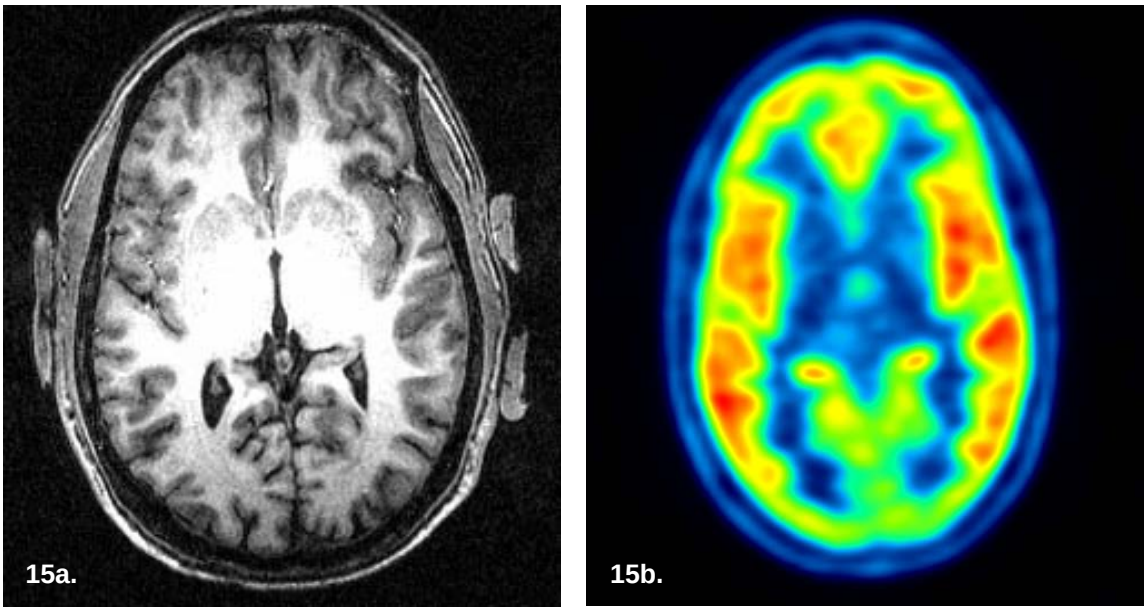

Figure 15a. **Individual T1-weighted MR image**

Figure 15b. **PET_{ADD} summation scan of a single subject.**

Red areas represent high radioactive activity and blue areas show regions with low activity.

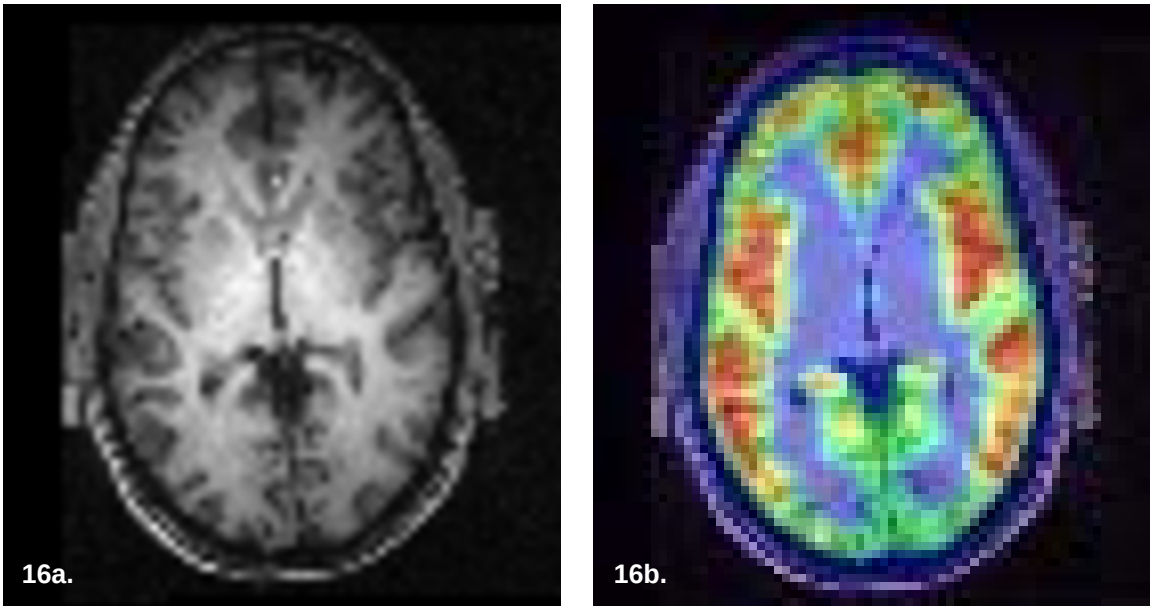

Figure 16a. **Co-registered MR image to PET_{ADD} summation scan of a single subject**

Figure 16b. **PET_{ADD} summation scan superimposed on the co-registered MR image**

Red areas represent high radioactive activity and blue areas show regions with low activity.

5.3.2. Regions of interest (ROIs)

Nine different brain areas were determined as ROIs (table 3) and delineated on the co-registered anatomical images using the VOI constructor tool of PMOD 2.55 and anatomical criteria established by Bremner et al. (Bremner et al. 1998).

Region of interest	**5-HT$_{1A}$ receptor density**
Hippocampus	high
Insula	high
Anterior cingulate cortex	high
Amygdala	high
Dorsal raphe nuclei	high
Orbitofrontal cortex	medium
Motor cortex	low
Visual cortex	low
Cerebellum	very low

Table 3. **Delineated ROIs and area specific 5-HT$_{1A}$ receptor density**
(Adapted from Hall et al. 1997)

Because of its low 5-HT$_{1A}$ receptor density (Burnet et al. 1997; Hall et al. 1997), the cerebellum was chosen as reference region. Due to the symmetry of the cerebellum it was delineated as one ROI over three slices on the co-registered MR image (see figure 17a,b).

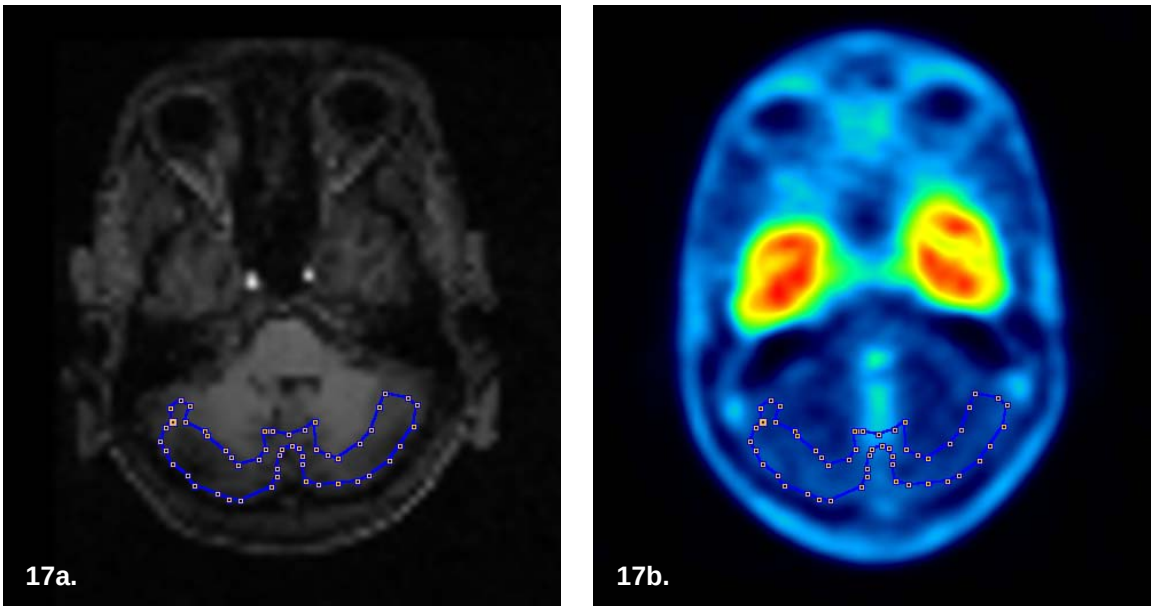

Figure 17a. **Cerebellum ROI of a single subject on the co-registered MR image**
Figure 17b. **Cerebellum ROI superimposed on the corresponding PET_{ADD} image**

The ROIs of hippocampus, amygdala, anterior cingulate cortex, insula, orbitofrontal cortex and the dorsal raphe nucleus have been reported as regions with high densities of 5-HT_{1A} receptors (Burnet et al. 1997; Hall et al. 1997; Pazos et al. 1987). All of these regions except for the dorsal raphe nucleus and the orbitofrontal cortex were delineated as different ROIs both in the left and the right hemisphere ranging over three slices (figure 18-22). Similar to the cerebellum, the orbitofrontal cortex was delineated as one ROI (figure 20).

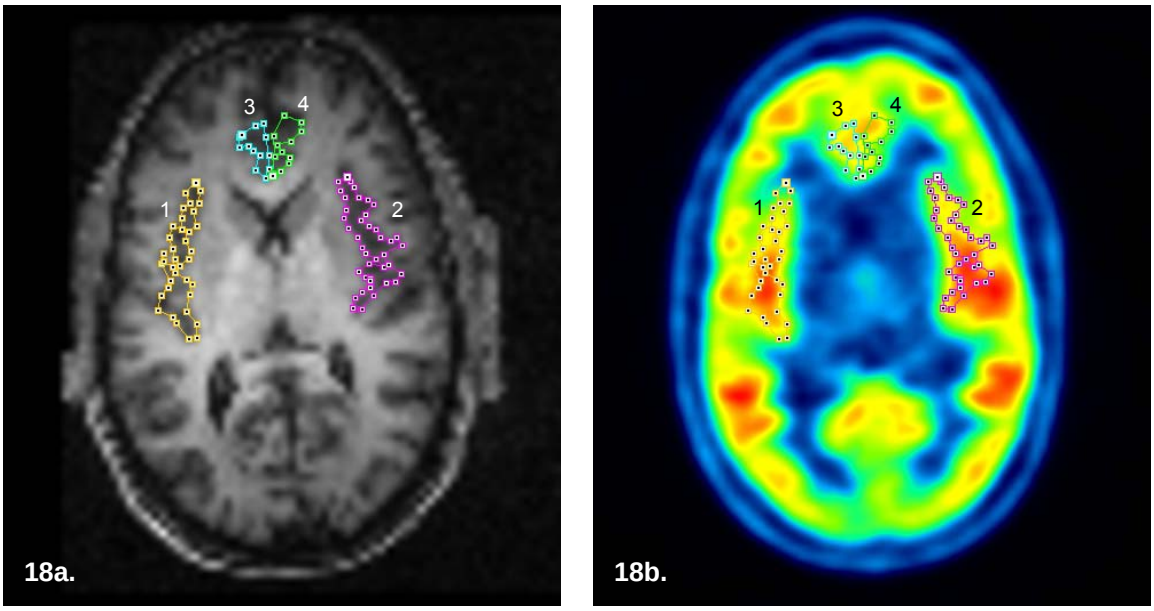

Figure 18a. **Individually delineated ROIs**

Delineated insula (1,2) and anterior cingulated cortex (3,4) ROIs on the co-registered MR image

Figrue 18b. **ROIs superimposed on the corresponding PET_{ADD} image**

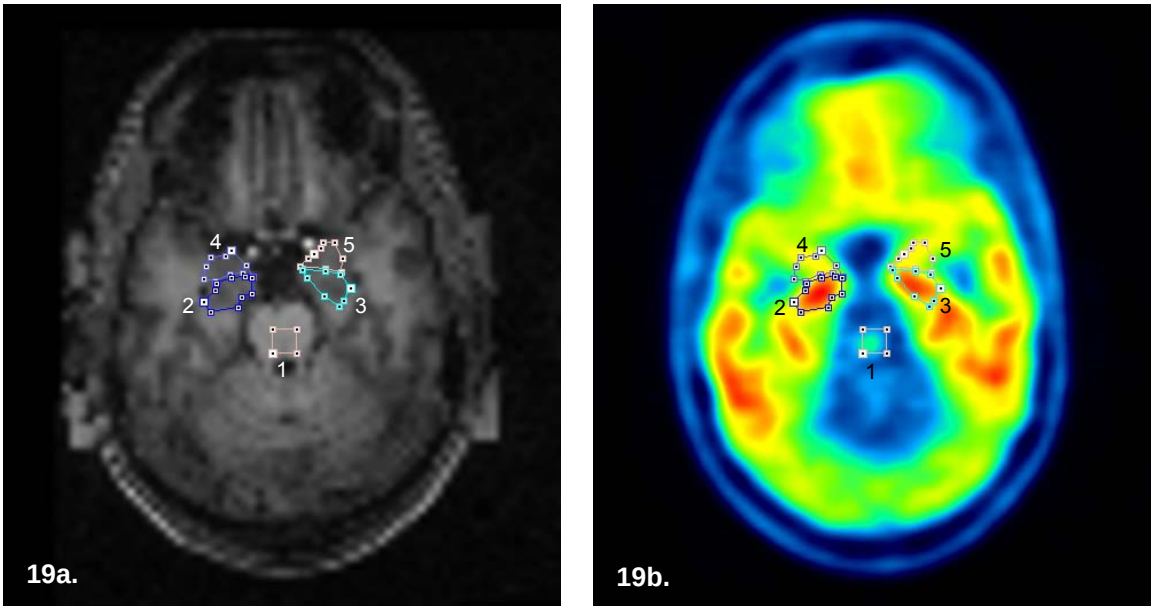

Figure 19a. **Individually delineated ROIs**

Dorsal raphe nucleus ROI (1) (Volume of 747 mm^3) and bilaterally delineated hippocampus (2,3) and amygdala (4,5) ROIs on the co-registered MR image.

Figure 19b. **ROIs superimposed on the corresponding PET_{ADD} image**

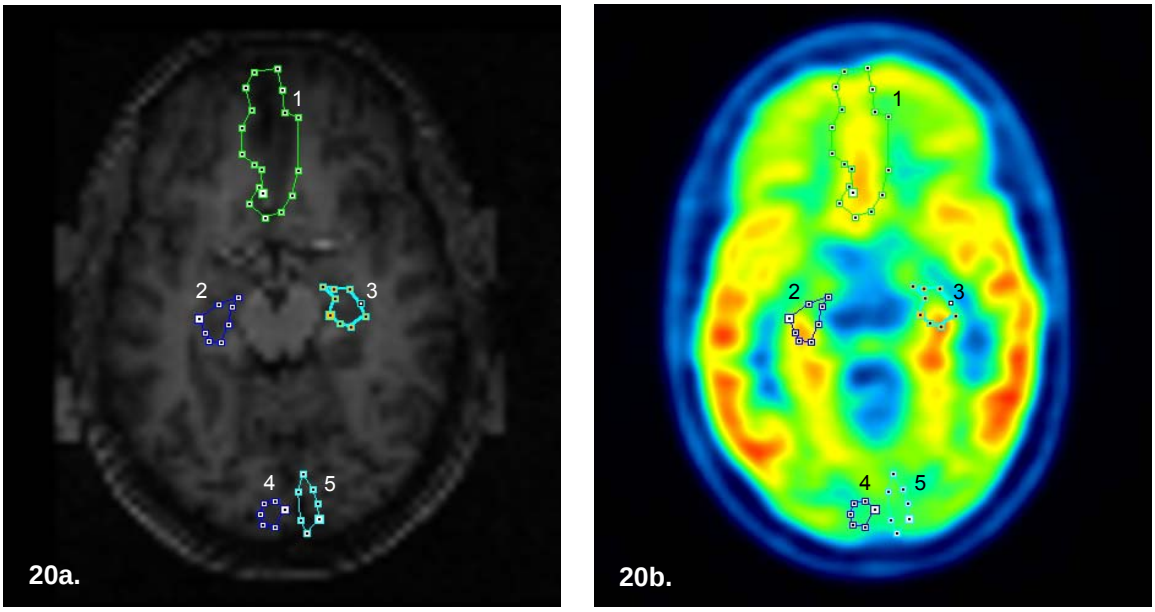

Figure 20a. **Individually delineated ROIs**

Orbitofrontal cortex ROI (1) and bilaterally delineated hippocampus (2,3) ROIs as well as visual cortex (4,5) ROIs on the co-registered MR image

Figure 20b. **ROIs superimposed on the corresponding PET_{ADD} image**

Due to the partial volume effect (PVE) resulting from the small dimensions (volume of 71.3 ± 4.5 mm^3) of the human dorsal raphe nucleus (Baker et al. 1990), an exact delineation of this region is hampered. Hence, the slices showing the interpeduncular cistern were identified on the co-registered MR image. On the corresponding slices of the PET_{ADD} a cubic ROI with a pre-assigned volume of 747 mm^3 was delineated (figure 19a,b) at the dorsal midbrain area containing high tissue signal (Tauscher et al. 2001b). The volume of 747 mm^3 was chosen for comparison with studies commonly assuming ROI sizes of this area ranging from 600 mm^3 (Tauscher et al. 2001b) to about 2000mm^3 (Parsey et al. 2002). The fact that the true dimensions of the dorsal raphe nuclei are much smaller than these volumes (Baker et al. 1990) consequently implies an underestimation of the 5-HT_{1A} RBP values when assuming too large ROI sizes. Therefore, ROIs with volumes of 42 mm^3, 83 mm^3 and 167 mm^3 were

delineated for obtaining more precise values of the 5-HT_{1A} receptor density (Spindelegger 2005). Figure 21 shows the relation of the delineated size optimized dorsal raphe nucleus ROIs superimposed on the PET_{ADD} image.

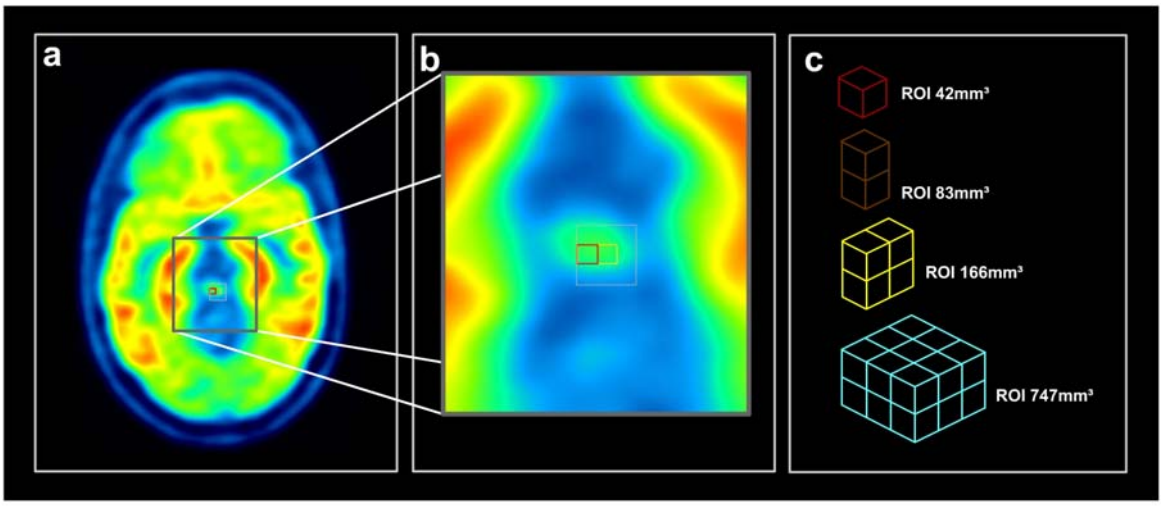

Figure 21a**. Different Raphe ROIs sizes superimposed on the PET_{ADD} image**
Figure 21b. **Enlargement of the dorsal raphe nucleus region**
(highlighted by a grey box in figure a) Raphe VOI 83 mm³ (brown); Raphe VOI 166 mm³ (yellow); Raphe VOI 747 mm³ (light blue); VOI of 42 mm³ is superimposed by the VOI of 83 mm³
Figure 21c. **The different delineated Raphe VOIs** (one cubus represents one voxel)
Raphe VOI 42 mm³ (red); Raphe VOI 83 mm³ (brown); Raphe VOI 166 mm³ (yellow); Raphe VOI 747 mm³ (light blue);

Furthermore, two regions with low receptor densities were delineated, namely motor cortex and visual cortex. The delineated ROIs of these regions are depicted in figure 20a,b (visual cortex ROIs) and 22a,b (motor cortex ROIs).

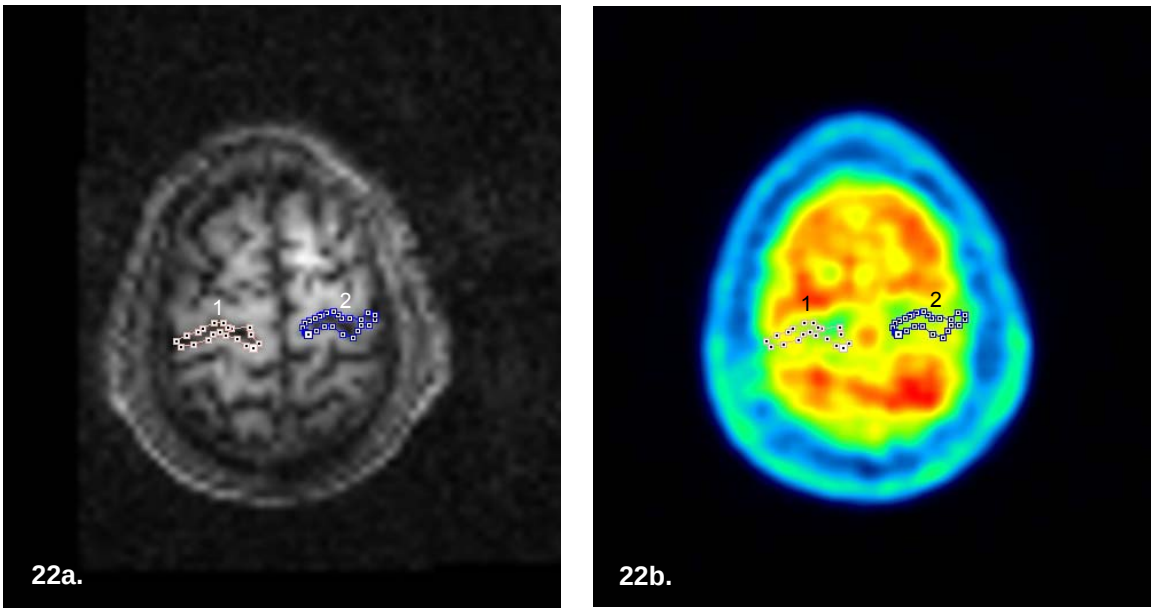

Figure 22a. **Individually delineated ROIs**

Bilaterally delineated motor cortex (1,2) ROIs on the co-registered MR image.

Figure 22b. **ROIs superimposed on the PET_{ADD} image**

5.3.3. Estimation of the 5-HT_{1A} receptor distribution applying PMOD 2.55

The estimation of receptor distribution was performed with the kinetic modelling tool of PMOD applying Lammertsma's simplified reference tissue model (SRTM) (Lammertsma and Hume 1996). Decay-corrected TACs (shown in figure 23) for each ROI were generated for the calculation of the regional 5-HT_{1A} RBPs with the VOI constructor tool of PMOD. Because the cerebellum is less of 5-HT_{1A} receptors (Burnet et al. 1997; Hall et al. 1997) the TAC of this region was chosen as reference function in the SRTM.

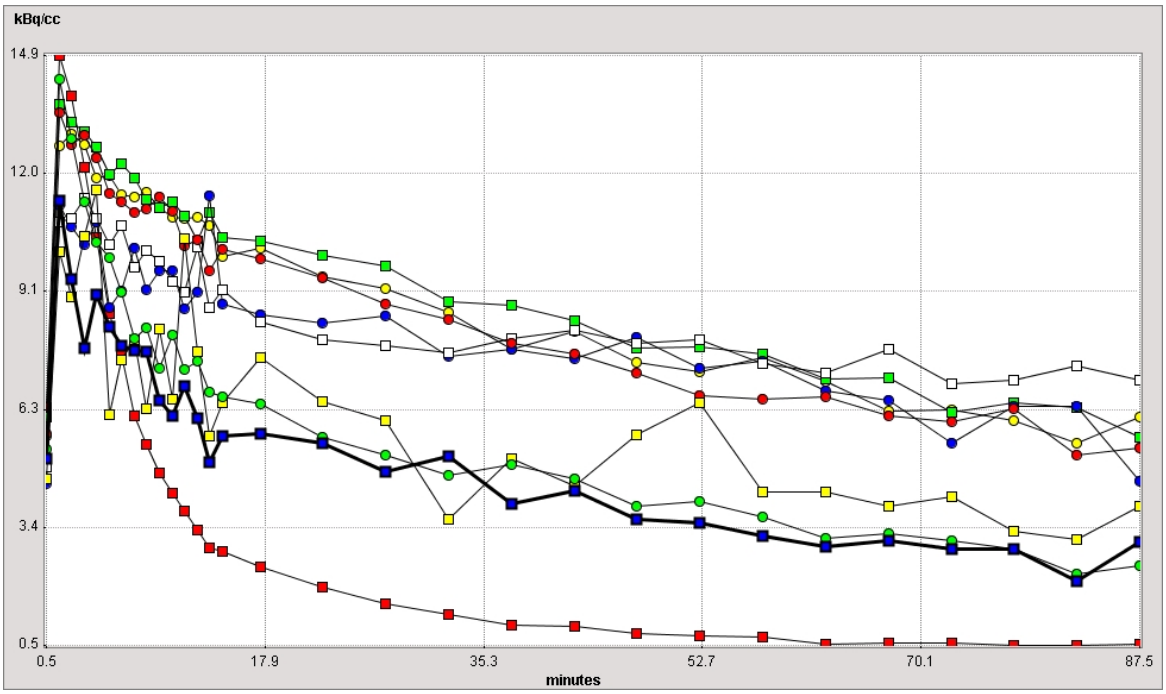

Figure 23. **Decay-corrected time activity curves (TACs) in delineated ROIs**

TACs of the delineated ROIs of the left hemisphere and the midbrain: insula (green squares), anterior cingulate (yellow circles), hippocampus (white squares), orbitofrontal cortex (red circles), amygdala (blue circles), dorsal raphe nucleus (yellow squares) (VOI 747 mm³), motor cortex (blue squares) and visual cortex (green circles), the reference region cerebellum (red squares).

5.3.4. Statistical analysis

The regional 5-HT_{1A} RBPs and the plasma levels of estradiol and progesterone were statistically analysed using SPSS 12.0.1 (© SPSS Inc. 2003). In case of bilaterally delineated ROIs, the mean 5-HT_{1A} RBP values of both sides were calculated. Mean and standard deviation of 5-HT_{1A} RBP values, the volume of each ROI and progesterone as well as estradiol plasma levels were calculated over the whole population.

Two-tailed Pearson (product-moment) correlation coefficients were generated for the demographic and methodological variables (age, BMI, cigarettes smoked per day, ROI volume and injected dose of [carbonyl-11C]-WAY-1006357/kg) with 5-HT_{1A} RBP of each ROI. Concerning steroid hormones, correlation of progesterone with the regional 5-HT_{1A} RBP was examined with two-tailed Pearson coefficients as well..

Multiple regression analyses including 5-HT_{1A} RBP as dependent variable and progesterone and age or progesterone, age and estradiol as independent variables were performed separately for each region using an alpha level of $p < 0.05$.

6. RESULTS

6.1. 5-HT_{1A} receptor binding potential

The 5-HT_{1A} receptor binding potential (RBPs) of seven postsynaptic and one presynaptic region are summarized in table 4. The volume of interest (VOI) ranges from 0.75 ± 0cm³ (pre-defined volume size of raphe nuclei) to 8.62 ± 2.20cm³ (orbitofrontal cortex).

Region of interest	**5-HT_{1A} RBP** mean ± SD	**VOI** mean ± SD [cm³]
Hippocampus	5.15 ± 1.61	3.16 ± 0.60
Insula	4.77 ± 1.18	5.96 ± 1.17
Anterior cingulate cortex	4.08 ± 0.95	2.18 ± 0.52
Amygdala	4.06 ± 1.12	1.64 ± 0.26
Orbitofrontal cortex	3.71 ± 0.88	8.62 ± 2.20
Motor cortex	2.17 ± 0.74	2.26 ± 1.07
Raphe nuclei	2.07 ± 0.57	0.75 ± 0
Visual cortex	1.81 ± 0.51	3.25 ± 0.68

Table 4. **5-HT_{1A} RBPs in different brain regions and the VOI of the delineated ROIs**
ROI listing according to the 5-HT_{1A} RBP values in descending rank order.

6.2. Plasma levels of steroid hormones

The means and standard deviations of the hormone plasma values are shown in table 5.

Hormone	Unit	Plasma levels mean ±SD	Reference range
Progesterone	ng/ml	1.0* ± 0.4	0-0.8
Estradiol	pg/ml	29.9 ± 8.0	14-60
Estradiol bioactive	pg/ml	19.4 ± 5.7	n.a.**

Table 5. **Progesterone and estradiol plasma values**

Mean hormone plasma values received from subjects' blood samples.

*Note: Since the subjects aged ranged between 25 and 52 years, the mean value of progesterone of this sample is higher than the reference values representing a broader age range.

**Note: reference range of bioactive estradiol not available.

6.3. Relationship of 5-HT_{1A} receptor BP with age, BMI and smoking habits

To investigate a relationship of subject characteristics and the smoking habits on 5-HT_{1A} RBP values, the variables age, body mass index (BMI) as well as the cigarettes smoked per day were correlated with the 5-HT_{1A} RBPs (see table 6,7).

Regions of interest	Age		BMI	
	Correlation coefficient	p	Correlation coefficient	p
Hippocampus	-0.06	0.804	-0.14	0.580
Insula	-0.26	0.292	-0.28	0.267
Anterior cingulate cortex	-0.20	0.436	-0.31	0.214
Amygdala	0.18	0.464	0.01	0.966
Orbitofrontal cortex	-0.19	0.451	-0.10	0.698
Motor cortex	-0.42	0.086	-0.20	0.423
Raphe nuclei	-0.08	0.749	-0.20	0.437
Visual cortex	-0.34	0.167	-0.37	0.128

Table 6. **Correlation of 5-HT_{1A} RBP and demographic variables (age, BMI)**
ROI listing according to the 5-HT_{1A} RBP values in descending rank order.

Consistent to the results of Rabiner et al. (Rabiner et al. 2002) who investigated 61 healthy subjects (35.5 ± 7.7 years, mean age ± SD), no significant correlation between the regional 5-HT_{1A} RBPs and age was found (for details see table 6). Furthermore, no significant correlation was detected between BMI and 5-HT_{1A} RBPs (table 6).

Benwell et al. reported an increase of 5-HT_{1A} receptors in the hippocampus of smokers (Benwell 1990). Similarly, 5-HT_{1A} RBPs were correlated with the number of cigarettes per day (table 7). No significant correlation between the number of cigarettes per day and the 5-HT_{1A} RBPs in the investigated ROIs was found.

Regions of interest	Cigarettes/day	
	Correlation coefficient	p
Hippocampus	0.03	0.918
Insula	0.08	0.767
Anterior cingulate cortex	0.04	0.872
Amygdala	-0.06	0.806
Orbitofrontal cortex	-0.05	0.854
Motor cortex	0.05	0.845
Raphe nuclei	0.09	0.736
Visual cortex	0.02	0.941

Table 7. **Correlation of 5-HT_{1A} RBP and cigarettes per day**
Cigarettes per day: 3.06 ± 4.41, mean ± SD; ROI listing according to the 5-HT_{1A} receptor BP values in descending rank order.

6.4. Relationship of 5-HT_{1A} RBP and the injected dose of [carbonyl-^{11}C]-WAY-100635

To exclude a significant relationship between the radiochemical variables and the 5-HT_{1A} RBPs in the delineated regions the regional RBPs were correlated with the injected dose of [carbonyl-^{11}C]-WAY-100635/kg and no significant correlation was obtained (range from r=-0.07 p=0.780 in the amygdala to r=0.25 p=0.312 in the insula; see table 8).

Regions of interest	Injected dose of [carbonyl-^{11}C]-WAY-100635/kg	
	Correlation coefficient	p
Hippocampus	0.05	0.850
Insula	0.25	0.312
Anterior cingulate cortex	0.24	0.343
Amygdala	-0.07	0.780
Orbitofrontal cortex	0.04	0.873
Motor cortex	0.25	0.326
Dorsal raphe nuclei	0.32	0.199
Visual cortex	0.32	0.202

Table 8. **Correlation of 5-HT$_{1A}$ RBP and injected dose of tracer**
Injected dose of [carbonyl-^{11}C]-WAY-100635/kg of 5.66 ± 0.85 MBq/kg, mean ± SD; ROI listing according to the 5-HT$_{1A}$ RBP values in descending rank order.

6.5. Correlation of progesterone and estradiol plasma levels with 5-HT$_{1A}$ RBPs

6.5.1. Presynaptic 5-HT$_{1A}$ autoreceptors in the dorsal raphe nuclei

In macaques, there was a decrease of the 5-HT$_{1A}$ autoreceptors mRNA in the dorsal raphe nuclei when treated with progesterone (Bethea et al. 2002; Lu and Bethea 2002; Pecins-Thompson and Bethea 1999). In humans, this study shows a significant negative correlation of progesterone plasma levels with 5-HT$_{1A}$ RBPs in this region (figure 24).

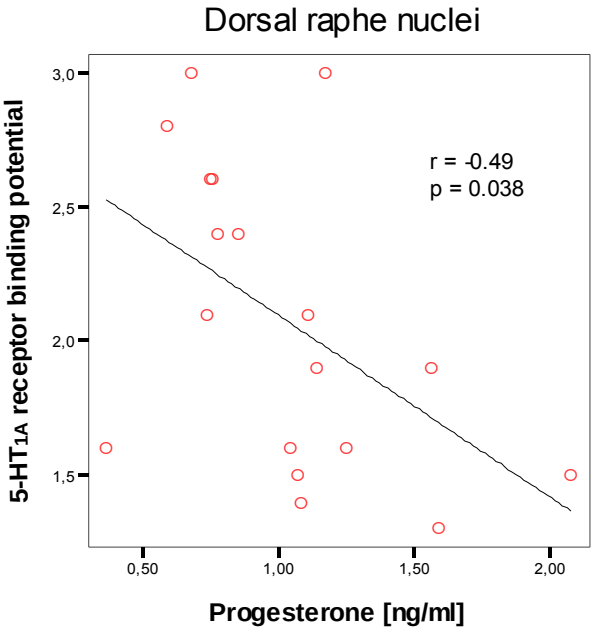

Figure 24. **Linear correlation of 5-HT_{1A} RBP with progesterone in the dorsal raphe nuclei**
The figure shows a significant negative correlation between the 5-HT_{1A} RBP of the dorsal raphe nuclei and the plasma levels of progesterone. Pearson's coefficient of r= -0.49 (p=0.038).

In ROIs of the dorsal raphe nucleus with smaller volumes (ranging from 42 mm³ to 167 mm³) a better fitting to progesterone plasma levels was found (Spindelegger 2005). Correlation coefficients of progesterone with the 5-HT_{1A} RBPs in the dorsal raphe nuclei when assuming different VOI are shown in table 9.

Dorsal raphe nuclei	5-HT_{1A} RBP	Progesterone	
VOI [mm³]	mean ± SD	Correlation coefficient	p
42	3.36 ± 0.96	**- 0.51***	0.032
83	2.87 ± 0.65	**- 0.56***	0.015
166	2.72 ± 0.63	**- 0.57***	0.013
747	2.07 ± 0.57	**- 0.49***	0.038

Table 9. **Correlation of 5-HT_{1A} RBP and progesterone plasma levels**
ROI listing according to the ROI volume in ascending rank order.
Note: *Significant correlation p< 0.05

Using a smaller VOI when delineating the dorsal raphe nuclei leads to a higher correlation between the plasma levels of progesterone and the 5-HT_{1A} RBPs. Based on these findings, the significance of the association between the 5-HT_{1A} RBPs in the raphe nuclei (dependent variable) and the independent variables progesterone and age was investigated by means of multiple regression analysis (table 10). Estradiol showed no significant correlation (r=0.06, p=0.807) with the 5-HT_{1A} RBPs in the dorsal raphe nuclei. There was no improvement of the significance levels when including estradiol in the multiple regression model (table 10, Model 2)

Dorsal raphe nucleus	Model 1 *Progesterone; Age*		Model 2 *Progesterone; Age; Estradiol*	
VOI [mm³]	adjusted r²	p	adjusted r²	p
42	**0.26***	0.039	0.23	0.086
83	**0.51***	0.002	**0.52***	0.004
166	**0.50***	0.002	**0.51***	0.005
747	**0.33***	0.019	0.29	0.053

Table 10. **Multiple regression analysis for the dorsal raphe nuclei**

Model 1: 5-HT_{1A} RBP (dependent variable) vs progesterone plasma levels (independent variable) and age (independent variable) of different VOIs of dorsal raphe nuclei.

Model 2: 5-HT_{1A} RBP (dependent variable) vs progesterone plasma levels (independent variable), age (independent variable) and estradiol plasma levels (independent variable) of different VOIs of dorsal raphe nuclei.

ROI listing according to the VOI in ascending rank order.

In order to test the hypothesis that smaller ROI sizes provide reliable data, the RBPs of the dorsal raphe nucleus region (different VOIs) were correlated with the RBPs of the cortical as well as subcortical regions mentioned before. Table 11 displays the intercorrelation of 5-HT_{1A} RBPs between the dorsal raphe nucleus and the other delineated regions. The highest intercorrelation coefficients and the best significance levels were found with the ROI volume of 166mm³.

	Dorsal raphe nucleus							
	VOI [mm³]		VOI [mm³]		VOI [mm³]		VOI [mm³]	
	42		**83**		**166**		**747**	
Region of interest	*Corr. coeff.*	*p*	*Corr. coeff.*	*p*	*Corr. coeff.*	*p*	*Corr. coeff.*	*p*
Anterior cingulate cortex	0.48*	0.0115	**0.68****	0.0017	***0.76*****	0.0002	**0.68****	0.0019
Insula	0.49*	0.0393	**0.66****	0.0032	***0.71*****	0.0010	**0.68****	0.0020
Motor cortex	0.45	0.0618	**0.61****	0.0077	**0.70****	0.0014	**0.66****	0.0031
Orbitofrontal cortex	**0.61****	0.0074	**0.70****	0.0013	**0.70****	0.0011	0.55*	0.0172
Amygdala	**0.61****	0.0072	**0.65****	0.0039	***0.72*****	0.0007	**0.60****	0.0089
Hippocampus	0.54*	0.0196	**0.63****	0.0052	**0.63****	0.0051	0.50*	0.0346
Visual cortex	0.31	0.2169	0.58*	0.0115	**0.66****	0.0031	**0.63****	0.0051

Table 11. **Intercorrelation of 5-HT$_{1A}$ RBPs between pre- and postsynaptic areas**
Intercorrelation between 5-HT$_{1A}$ RBPs of the dorsal raphe nucleus (different VOI sizes) and 5-HT$_{1A}$ RBPs in cortical as well as subcortical regions. Corr. coeff.: Correlation coefficient.

Note: * Significant correlation p< 0.05
** High significant correlation p<0.01
*** Very high significant correlation p< 0.001

6.5.2. Postsynaptic 5-HT$_{1A}$ receptor binding potentials

In contrast to the raphe region, 5-HT$_{1A}$ receptors in cortical regions and the limbic system are located postsynaptically, mostly on glutamatergic or GABAergic neurons. There are also receptors for estradiol and progesterone located within these neurons and several studies indicate an effect of these hormones on the expression of the postsynaptic 5-HT$_{1A}$ receptors (Bethea et al 2002; Alper et al. 2000). The following table (table 12) shows the correlation coefficients and significance levels between 5-HT$_{1A}$ RBPs and progesterone plasma levels in cortical and subcortical ROIs.

Region of interest	Progesterone	
	Correlation coefficient	p
Hippocampus	-0.46	0.058
Insula	-0.30	0.222
Anterior cingulate cortex	-0.34	0.163
Amygdala	**-0.62***	0.006
Orbitofrontal cortex	-0.43	0.077
Motor cortex	-0.16	0.540
Visual cortex	-0.17	0.508

Table 12. **Correlation of 5-HT_{1A} RBPs and progesterone plasma levels**
ROI listing according to the 5-HT_{1A} RBP values in descending rank order.
Note: * Significant correlation $p < 0.05$

As depicted in table 12 only the 5-HT_{1A} RBPs of the amygdala region show high significant correlation with progesterone plasma levels (r=0.62, p=0.006) (figure 25), whereas the 5-HT_{1A} RBPs of the hippocampus regions show a trend towards significant correlation (r=0.46, p=0.058, figure 26). All other specified regions did not show a significant correlation when analysis was restricted to progesterone without age correction.

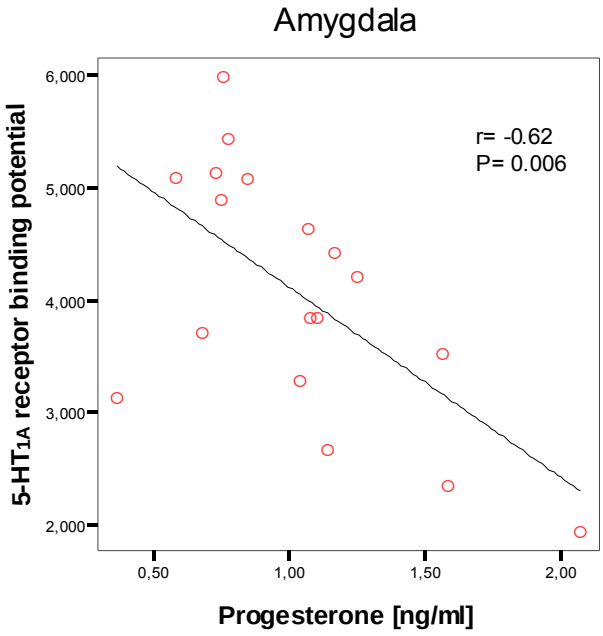

Figure 25. **Linear correlation of 5-HT$_{1A}$ RBP with progesterone in the amygdala**

The figure displays a highly significant negative correlation between the 5-HT$_{1A}$ RBP of the amygdala and the plasma levels of progesterone. Pearson's coefficient of r= -0.62, p=0.006.

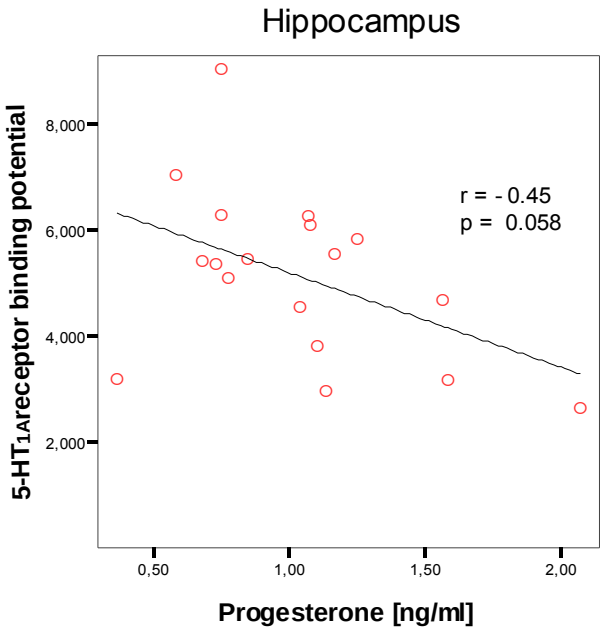

Figure 26. **Linear correlation of 5-HT$_{1A}$ RBP with progesterone in the hippocampus**

The figure displays a borderline significant negative correlation between the 5-HT$_{1A}$ RBP of the hippocampus region and the plasma levels of progesterone. Pearson's coefficient of r= -0.45, p=0.058.

A correlation of age and progesterone plasma levels yields a significant age related change of progesterone plasma levels (r=-0.538, p=0.021). A multiple regression analysis with the 5-HT_{1A} RBPs as dependent and progesterone and age as independent variables was done (table 13). Details of the multiple regression analysis including 5-HT_{1A} RBPs as dependent, progesterone, age and estradiol as independent variables are given in table 13.

	Model 1 Progesterone; Age		**Model 2** Progesterone; Age; Estradiol	
Regions of interest	adjusted r^2	p	adjusted r^2	p
Hippocampus	**0.25***	0.044	**0.33***	0.036
Insula	**0.26***	0.041	0.29	0.051
Anterior cingulate cortex	0.23	0.054	**0.31***	0.042
Amygdala	**0.34***	0.018	**0.41***	0.016
Orbitofrontal cortex	**0.35***	0.015	**0.38***	0.022
Motor cortex	**0.29***	0.030	0.27	0.060
Visual cortex	0.19	0.078	0.19	0.115

Table 13. **Multiple regression analysis Model 1 and Model 2**

Model 1: 5-HT_{1A} RBP (dependent variable) vs progesterone plasma levels (independent variable) and age (independent variable) of different cortical and subcortical ROIs.

Model 2: 5-HT_{1A} RBP (dependent variable) vs progesterone plasma levels (independent variable), age (independent variable) and estradiol plasma levels (independent variable) of different cortical and subcortical ROIs. ROI listing according to the 5-HT_{1A} RBP values in descending rank order.

Note: * Significant correlation $p < 0.05$

A correction for age (model 1, table 13) improved the significance levels in all regions except for the amygdala. Including progesterone, age and estradiol in the model 2 (table 13) significance levels for hippocampus, anterior cingulate cortex and amygdala were improved.

7. DISCUSSION

Several studies have reported a relationship between serotonergic neurotransmission, steroid hormones and affective states in physiological and pathological conditions in animals (Bethea et al. 2002; Bethea et al. 2005; Blier and de Montigny 1999; Drevets et al. 1999; Gross et al. 2002; Neumeister et al. 2004; Pecins-Thompson and Bethea 1999; Robichaud and Debonnel 2004; Tauscher et al. 2001a). To my best knowledge, this is the first investigation in humans showing a relationship of the steroids hormones progesterone and estradiol plasma levels and the 5-HT_{1A} receptor binding potential (RBP) in the human brain.

Bethea et al. described the influence of progesterone and estradiol on the 5-HT_{1A} receptor density in the dorsal raphe nuclei as well as in cortical and subcortical brain regions in non-human primates (Bethea et al. 2000; Bethea et al. 2002; Pecins-Thompson and Bethea 1999; Robichaud and Debonnel 2004; 2005). Based on these studies the following hypotheses were tested in healthy males *in vivo:*

1. Progesterone and estradiol plasma levels correlate with the presynaptic 5-HT_{1A} autoreceptor binding potential in dorsal raphe nucleus.

2. Progesterone and estradiol plasma levels correlate with the postsynaptic 5-HT_{1A} receptor binding potential in several cortical and subcortical areas.

3. The influence of progesterone and estradiol is significantly pronounced in regions expressing high levels of steroid hormone receptors in cortical and subcortical areas.

To verify the first hypothesis, the plasma levels of progesterone and the 5-HT_{1A} RBPs in the dorsal raphe nuclei were correlated with different ROI sizes and a significant negative correlation was found (figure 24, table 9). These findings comply with results from investigations in macaques that showed a decrease of the 5-HT_{1A} autoreceptors mRNA in the dorsal raphe nuclei when treated with progesterone (Bethea et al. 2002; Lu and Bethea 2002; Pecins-Thompson and Bethea 1999).

Due to a significant decrease of progesterone with age in our subjects (r=-0.538 p=0.021), a multiple regression analysis with the 5-HT_{1A} RBPs as dependent and progesterone and age as independent variables was performed. There was a highly significant association between these variables (table 10). Estradiol mediates progesterone receptor expression in the dorsal raphe nuclei (Bethea 1994; Bethea et al. 2002), therefore estradiol as an additional independent variable was included in the mentioned multiple regression.

As far as the true extensions of the dorsal raphe nuclei (71.3 ± 4.5 mm^3, mean volume ± SD) are concerned (Baker et al. 1990), the majority of studies uses too large ROI sizes ranging from 600 mm^3 (Tauscher et al. 2001b) to about 880 ± 742 mm^3 (Parsey et al. 2000) causing an underestimation of 5-HT_{1A} RBPs in this region. Therefore, the multiple regression analyses including progesterone, age and estradiol as independent variables, were performed with different ROI sizes of the dorsal raphe nucleus as dependent variable. A fixed ROI volume of 83 mm^3 or 166 mm^3 yields highly significant results, whereas an ROI volume of 747 mm^2 do not show a significant fit of the model (table 10). These results concerning the ROI sizes, are similar to previously reported findings (Spindelegger et al. 2005) and suggest that smaller ROI sizes of the dorsal raphe nucleus provide more accurate results. To conclude, our data support the first hypothesis showing a significant negative correlation of 5-HT_{1A} autoreceptors in the dorsal raphe nuclei with progesterone plasma levels. These data indicate a steroid-

hormone-dependent regulation mechanism of presynaptic 5-HT_{1A} autoreceptors in humans as shown in non-human primates.

This investigation reveals a highly significant negative correlation of postsynaptic 5-HT_{1A} RBPs in cortical areas and the limbic system with progesterone plasma levels only in the amygdala region (table 12; figure 25) supporting the second hypothesis. In contrast, the 5-HT_{1A} RBPs of other regions showed no significant correlation with progesterone. The inclusion of progesterone, estradiol and age into a multiple regression analysis as independent variables yields in an improvement of the significance values of the regression model in the orbitofrontal cortex, the anterior cingulate cortex and the limbic areas hippocampus, amygdala compared to the regression analysis including progesterone and age as independent variables (table 13). These results support the third hypothesis of pronounced influence of both progesterone and estradiol on the postsynaptic 5-HT_{1A} receptor density in brain regions with high levels of steroid hormone receptors (Gundlah et al. 2000; Levine et al. 2001; Osterlund et al. 2000a; Osterlund et al. 2000b).

Our results showed a negative correlation between progesterone and estradiol plasma levels and the inhibitory postsynaptic 5-HT_{1A} receptors in several cortical regions as well as in the limbic system. When comparing these results to findings of a steroid hormone induced change of the excitatory 5-HT_{2A} receptor density on glutaminergic and GABAergic neurons (Amargos-Bosch et al. 2004; Kugaya et al. 2003; Moses et al. 2000), these data suggest a shift of the balance between the serotonergic inhibitory and excitatory effects on these neurons mediated by steroid hormones. Concluding from that, progesterone and estradiol seem to have a strong impact on the serotonergic neurotransmission. Therefore, these findings could provide a biological rationale to explain gender differences in psychiatric disorders linked to changes of the serotonergic system.

This study yielded similar 5-HT_{1A} receptor distributions as reported by post-mortem autoradiography studies (Burnet et al. 1997; Hall et al. 1997) and in vivo studies with [carbonyl-^{11}C]-WAY-100635 (Rabiner et al. 2002). Regarding the delineated ROIs we are in line with the rank order in the findings of Rabiner (Rabiner et al. 2002) who investigated the 5-HT_{1A} RBPs of a large group (61 subjects) of healthy male volunteers. As mentioned in chapter 6.3. and 6.4., any significant ($p< 0.05$) bias of selected demographic (age, BMI), smoking habits or methodological (VOI, radiochemicals) variables on the 5-HT_{1A} RBPs (table 6-8) were exlcluded.

Referring to the correlation between age and regional 5-HT_{1A} RBPs, contradictory results can be found in the literature. The post-mortem study of Dillon (Dillon et al. 1991) revealed an age related decrease in 5-HT_{1A} receptor density. Following that, Meltzer (Meltzer et al. 2001) reported a significant negative correlation between age and 5-HT_{1A} RBPs in different cortical and subcortical regions including the raphe nuclei in healthy volunteers, whereas Tauscher (Tauscher et al. 2001b) detected a negative correlation of regional 5-HT_{1A} RBPs and age only in cortical regions. In contrast to these findings, several other studies with samples sizes ranging from six (Farde et al. 1998; Gunn et al. 1998) to 61 (Rabiner et al. 2002), did not found an age-related change of the 5-HT_{1A} RBP in cortical regions and in the raphe nuclei of human subjects. Our results in healthy males are in line with the findings of Rabiner et al. who investigated a large group of volunteers showing no age effect on the 5-HT_{1A} density. The broad diversity of results can be attributed to the various sample sizes and different age ranges in the studies mentioned.

The findings of this study revealed on the one hand a significant influence of progesterone on the 5-HT_{1A} RBP especially in the dorsal raphe nuclei and on the other hand an age-related decrease of progesterone plasma levels. Contrary, no significant

correlation of the 5-HT_{1A} RBPs with rising age were found. Thus, the data suggest that the 5-HT_{1A} receptor distribution is modulated by the relation between progesterone and estradiol plasma levels. This assumption is supported by the improvement of the significance level (table 13) in several cortical as well as subcortical regions when including estradiol as an additional variable into the regression model and the findings of estrogen induced PR in various brain areas (Bethea 1994; Parsons et al. 1980; Romano et al. 1989).

Regarding the BMI of the subjects, this study did not show any influence on the 5-HT_{1A} RBPs. These findings are congruent with the results of Rabiner (Rabiner et al. 2002) who did not find a significant correlation between weight or height and the 5-HT_{1A} RBPs in the cortex or the raphe nuclei.

An interaction of smoking and the serotonergic system was investigated in a human post-mortem study by Benwell (Benwell et al. 1990) who detected an increase of 5-HT_{1A} receptors in the hippocampus of smokers. Another relationship between nicotine and the 5-HT_{1A} receptors was reported by Rasmussen, who described the pivotal role of 5-HT_{1A} receptors in the neurophysiology of nicotine withdrawal (Rasmussen et al. 1997). Furthermore, 5-HT_{1A} receptors in the dorsal raphe nuclei mediate some of the psychotropic effects of nicotine (Cheeta et al. 2001; Mihailescu et al. 2001). Kenny (Kenny et al. 2001) indicated the regulatory role of nicotine on the 5-HT_{1A} receptors gene expression in several cortical regions especially in the dorsal hippocampus. Therefore, we investigated the correlation between the cigarettes smoked per day and the 5-HT_{1A} RBPs. The lack of significant correlation coefficients between the 5-HT_{1A} receptor distribution and the cigarettes smoked per day can probably be traced to the fact that no heavy smokers (3,06 ±4,41, mean cigarettes per day ±SD) took part in our study (table 7).

To exclude the bias of radiochemical variables the injected dose of [carbonyl-^{11}C]-WAY-100635/kg body weight were correlated with the regional 5-HT_{1A} RBPs (table 8) and found no significant correlation between these variables. The injected dose of [carbonyl-^{11}C]-WAY-100635 in this investigation yielded comparable 5-HT_{1A} RBP levels than Rabiner et al. who used lower doses (Rabiner et al. 2002). Therefore, that indicates no relationship between the injected dose of the tracer and the regional 5-HT_{1A} RBP.

VOIs were correlated with the regional 5-HT_{1A} RBP to exclude any impact of the VOI on the 5-HT_{1A} receptor density. Since no significant correlation except for the dorsal raphe nuclei region were found, the applied VOIs in this study do not exert any effects on the estimated 5-HT_{1A} RBPs. According to the different VOI delineated for the dorsal raphe nucleus, the smallest VOI (size 42mm³) leads to the highest 5-HT_{1A} RBPs. Probably, due to movement artifacts and the partial volume effect (PVE), 5-HT_{1A} RBPs of in the smallest VOI did not achieve the highest correlation coefficients with progesterone, age and estradiol. An intercorrelation of 5-HT_{1A} RBPs in the dorsal raphe nuclei (different ROI volumes) with the 5-HT_{1A} RBPs in cortical regions and the limbic system also indicated the optimum VOI between 83mm³ and 166mm³ (table 11). These findings are in line with the results of Baker et al. who investigated the volume of the dorsal raphe nucleus (71.3 ±4.5mm^3 mean volume ±SD) in healthy human males (Baker et al. 1990). The accuracy of small ROI sizes offers the opportunity to delineate small regions containing high 5-HT_{1A} receptor levels more precisely. This enables a better distinction of the subnuclei in various brain regions, e.g. the hypothalamus.

8. CONCLUSION AND RELEVANCE TO PSYCHIATRY

To my best knowledge, these data reveals for the first time a significant relationship between the sex steroid plasma levels for progesterone and estradiol and the serotonin-1A (5-HT_{1A}) receptor densities in the human brain *in vivo.* Our data are consistent to studies done by Bethea et al. showing a modulation mechanism of progesterone and estradiol on the 5-HT_{1A} receptor densities in non-human primates (Bethea et al. 2000; Bethea et al. 2002; Pecins-Thompson and Bethea 1999) and Krezel in rodents (Krezel et al. 2001). The findings suggest that changes of steroid hormone plasma levels could cause a shift in the balance between the serotonergic inhibitory and excitatory activity on glutaminergic and GABAergic neurons. Consequently, this shift to the serotonergic excitatory modulation of these neurons could imply a higher vulnerability to affective disorders (Krezel et al. 2001; Sibille et al. 2000). Gender differences in the prevalence to depression and anxiety disorders as well as disturbances in the hypothalamus-pituitary-adrenal/gonadal axis (HPA/HPG) were frequently reported (Angold and Worthman 1993; Baischer et al. 1995; Halbreich et al. 1995; Kornstein et al. 2000; Meller et al. 2001; Rubin et al. 1989; Steiner et al. 2003). Futhermore, several studies indicated an improvement in symptoms of postpartum depression (Ahokas et al. 2001; Galea et al. 2001) as well as in mild depression in postmenopausal women (Cohen et al. 2003; Soares et al. 2001) by treatment with estradiol. In addition, sex differences in the response to antidepressants combined with sex steroids were found in sheeps (Soares et al. 2001). The main results of this study support the assumption of an essential regulatory mechanism of the serotonergic neurotransmission by the steroid hormones progesterone and estradiol in humans. This could provide biological rationale for gender differences in affective disorders and the improvement of symptoms in patients suffering from depression when treated with estradiol or gestagens. Following this, a gender

specific treatment or an augmentation approach with steroid hormones in patients suffering from depression or anxiety disorders might improve the clinical outcome.

9. FIGURES

10. TABLES

11. ABBREVIATIONS

5-HT	Serotonin
5-HT_{1A}	Serotonin-1A receptor
5-HT_{2A}	Serotonin-2A receptor
5-HIAA	5-hydroxyindoleacetic acid
B_{max}	Maximum concentration of binding sites
BMI	Body Mass Index
DNA	Deoxyribonucleic Acid
DRN	Dorsal raphe nucleus
ECG	Electrocardiogram
ECLIA	Electrochemiluminescence Immunoassay
ER	Estradiol receptor
ERα	Estrogen receptor type α
ERβ	Estrogen receptor type β
f_2	Free fraction of the unbound tracer
f_i	Free fraction of the competing endogenous ligand
FWHM	Full Width at Half Maximum
HPA/HPG	hypothalamus-pituitary-adrenal /gonadal axi
HPLC	High performance liquid chromatography
K_D	Dissociation constants for the radiotracer
keV	Kilo Electron Volt
K_i	Dissociation constants for the competing endogenous ligand
LOR	Line of response
MAO_A	Monoaminooxidase type A
MAO_B	Monoaminooxidase type B
MBq	Megabecquerel
MINI	Mini International Neuropsychiatric Interview
MR image	Magnetic resonance image
MRN	Median raphe nucleus
mRNA	Messenger Ribonucleic Acid
PET	Positron Emission Tomography
PR	Progesterone receptor
PR_A	Progesterone receptor type A
PR_B	Progesterone receptor type B
RBP	Receptor binding potential
ROI	Region of interest
SERT	Serotonin transporter
SHBG	Sex Hormone Binding Globulin
SLN	Supralemniscal nucleus
SPECT	Single Photon Emission Computed Tomography
SRTM	Simplified Reference Tissue Model
STAI	Spielberger State Trait Anxiety Inventory
TAC	Time Activity Curve
THF	Tetrahydrofuran
VOI	Volume of interest

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