Diplomarbeit

Serotonin-1A receptor distribution in pre- and postmenopausal women quantified by PET and [carbonyl-^{11}C]WAY100635

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SUMMARY

BACKGROUND

Several studies on rodents and non-human primates indicate an influence of steroidal hormones on serotonergic neurotransmission. Despite these findings little is known about their effects on the human brain, in particular only a few studies were conducted investigating their influence on serotonin-1A receptors (5-HT1AR), the main inhibitory receptors of the serotonergic system.
This study aimed to depict the effect of menopausal status ("premenopausal" vs. "postmenopausal") on the nondisplaceable binding potential (BPND) of the serotonin-1A receptor in pre- and postmenopausal women by means of positron emission tomography (PET). Additionally, this study investigated if a correlation between plasma levels of steroid hormones and the 5-HT1A receptor binding potential can be traced in a subject sample of healthy pre- and postmenopausal women.

METHODS

A total of 43 healthy women (premenopausal n=18, 24.11 ± 2.58 years [mean ± SD]; postmenopausal n=25, 55.36 ± 4.76 years [mean ± SD]) underwent a PET scan with the highly selective radioligand [carbonyl-^{11}C]WAY100635. Quantification of the 5-HT1A binding potential (BPND) was done in 14 a priori defined regions of interest (Superior temporal gyrus, Middle temporal gyrus, Inferior temporal gyrus, Insula, Fusiform gyrus, Olfactory cortex, Gyrus rectus, Caput hippocampi, Amygdala, Hippocampus, Parahippocampal gyrus, Anterior cingulate cortex, Dorsal raphe nucleus, Median raphe nucleus) and cerebellum as reference region using PMOD 3.1 and applying the Simplified reference tissue model 2 (SRTM2). Blood samples of the steroidal hormones 17ß-estradiol, progesterone, testosterone as well as bioavailable testosterone were collected prior to PET measurement.

RESULTS

A mixed model ANOVA could not trace a significant effect of menopausal status on the dependent variable 5-HT1A BPND in this subject sample of 43 females [F (1, 41,756) =.078, p=.0781]. The post-hoc analysis in each region of interest (ROI) by means of t-tests and applying Bonferroni correction for multiple comparisons with an adjusted p=.003 did not reveal significant differences in BPND between pre- and postmenopausal women. In the group of postmenopausal women an inverse relationship between plasma levels of bioavailable testosterone and 5-HT1A BPND in all investigated ROIs was detected. Ten out of fourteen ROIs survived the Bonferroni correction for multiple comparisons (adjusted p=.003), namely olfactory cortex (r= -0.604, p=.002), fusiform gyrus (r= -0.604, p=.002), superior temporal gyrus (r=-0.613, p=.002), anterior cingulate cortex (r=-0.635, p=.001), hippocampus (r=-0.596, p=.003), parahippocampal gyrus (r=-0.584, p=.003), insula (r=-0.604, p=.002), amygdala (r=0.590, p=.003), dorsal raphe nucleus (r=-0.618, p=.002) and median raphe nucleus (r=-0.652, p=.001). Correlations between testosterone and BPND were also significant in all investigated regions, but only the dorsal raphe nucleus survived the Bonferroni correction (r=-0.577, p=.003).

CONCLUSION

The validity of the result of the mixed model ANOVA is limited due to the fact that "age" could not be partialled out as a covariate and should be interpreted with caution.

Apart from that this study provides the first evidence of a suppressive effect of testosterone and bioavailable testosterone on 5-HT1A RBP in humans. However, this finding stands in contrast to a study in premenopausal women which detected a positive correlation between these two variables. Considering diverging animal study results as well, this question needs to be reexamined. Longitudinal studies with steroid hormone administration should be conducted to elucidate these interrelations. There is a definite need for future trials that take into account the causation of large inter-subject variabilities by means of identification of subgroups (e.g. differences due to genetic polymorphisms). A separate comparison of these subjects would help us to establish a greater degree of accuracy on this matter and will add substantially to our understanding of the influence of steroidal hormones on serotonergic neurotransmission.

ZUSAMMENFASSUNG

HINTERGRUND

Eine Vielzahl von Studien an Nagetieren und nicht-menschlichen Primaten deutet darauf hin, dass Steroidhormone Einfluss auf das serotonerge System haben. Trotz dieser Erkenntnisse ist über deren Wirkung auf das menschliche Gehirn nur wenig bekannt, im Besonderen wurden nur wenige Studien durchgeführt, die deren Wirkung auf Serotonin-1A-Rezeptoren (5-HT1AR) untersuchen.

Das Ziel dieser Studie war es zu untersuchen, ob die Zugehörigkeit zu einer der beiden Lebensphasen „prämenopausal vs. „postmenopausal" einen Effekt auf das Bindungspotential (BPND) der 5-HT1A-Rezeptoren in einem Probandenkollektiv von prä- und postmenopausalen Frauen hatte. Zusätzlich wurde untersucht, ob die Höhe der Plasmaspiegel von Steroidhormonen der Probandinnen mit der Höhe des 5-HT1AR-Bindungspotentials korrelierte.

METHODEN

Die Studiengruppe umfasste insgesamt 43 gesunde Probandinnen (prämenopausal n=18, 24.11 ± 2.58 Jahre [Mittelwert ± Standardabweichung]; postmenopausal n=25, 55.36 ± 4.76 Jahre [Mittelwert ± Standardabweichung]). Alle Probandinnen unterzogen sich einer Positronen-Emissions-Tomographie-Untersuchung unter Anwendung des selektiven Radioliganden [carbonyl-^{11}C]WAY100635. Die Quantifizierung des 5-HT1AR-BPND erfolgte in 14 a priori festgelegten Zielregionen (Gyrus temporalis superior, Gyrus temporalis medius, Gyrus temporalis inferior, Insula, Gyrus fusiformis, Olfaktorischer Kortex, Gyrus rectus, Caput hippocampi, Amygdala, Hippocampus, Gyrus parahippocampalis, anteriorer Gyrus cynguli, Nucleus raphe medianus, Nucleus raphe dorsalis) und dem Cerebellum als Referenzregion mittels PMOD 3.1 und unter Anwendung des „Simplified Reference Tissue Model 2" (SRTM2). In den vor der PET-Messung entnommenen Blutproben wurden die Plasmaspiegel der Streoidhormone 17ß-estradiol, Progesteron, Testosteron, als auch bioverfügbares Testosteron bestimmt.

ERGEBNISSE

Anhand des gemischten Modells der ANOVA konnte kein signifikanter Effekt der Zugehörigkeit zu einer der beiden Lebensphasen „prämenopausal vs. „postmenopausal" auf die Höhe des 5-HT1A-BP in dieser Stichprobe von 43 weiblichen Probanden nachgewiesen werden [F (1, 41,756) =.078, p=.0781]. Jede ROI wurde post-hoc mit Hilfe von t-Tests untersucht und es wurde eine Bonferroni-Korrektur bei multiplen Paarvergleichen mit einem angepassten Signifikanzniveau von p=.003 angewendet. Es konnten keine signifikanten regionalen Unterschiede im BPND zwischen prä- und postmenopausalen Frauen gefunden werden. In der Gruppe der postmenopausalen Probandinnen konnte eine negative Korrelation zwischen der Höhe der Plasmaspiegel von bioverfügbarem Testosteron und dem 5-HT1ARBP in allen untersuchten Regionen nachgewiesen werden. Zehn von vierzehn dieser Regionen bestanden die Bonferroni-Korrektur (adjustiertes p=.003), darunter Olfaktorischer Kortex (r= -0.604, p=.002), Gyrus fusiformis (r= -0.604, p=.002), Gyrus temporalis superior (r=-0.613, p=.002), anteriorer Gyrus cynguli (r=-0.635, p=.001), Hippocampus (r=-0.596, p=.003), Gyrus parahippocampalis (r=-0.584, p=.003), Insula (r=-0.604, p=.002), Amygdala (r=-0.590, p=.003), Nucleus raphe dorsalis (r=-0.618, p=.002), als auch der Nucleus raphe medianus (r=-0.652, p=.001). Auch die Plasmaspiegel von Testosteron und die 5-HT1A Bindungspotentiale wiesen eine

signifikante negative Korrelation in allen untersuchten Regionen auf, jedoch blieb nach Bonferroni-Korrektur nur der Nucleus raphe dorsalis significant (r=-0.577, p=.003).

SCHLUSSFOLGERUNG

Die Aussagekraft des Ergebnisses der Gemischte-Modelle-ANOVA ist limitiert durch die Tatsache, dass das „Alter“ nicht als Kovariate herausgerechnet werden konnte und sollte deshalb mit Vorsicht interpretiert werden.

Abgesehen davon liefert diese Studie erstmals einen Hinweis auf einen suppressiven Effekt von Testosteron und bioverfügbaren Testosteron auf das 5-HT1A RBP beim Menschen. Dieses Ergebnis steht jedoch im Widerspruch zu einer Studie an prämenopausalen Frauen, in der ein positiver Zusammenhang zwischen diesen zwei Variablen gefunden wurde. In Anbetracht ebenfalls widersprüchlicher Ergebnisse in Tierstudien muss diese Fragestellung in weiteren Untersuchungen nachgeprüft werden. Longitudinale Studien mit Administration von Steroidhormonen sollten durchgeführt werden, um diese Wechselbeziehungen aufzuklären. Es gibt definitiv Bedarf an Studien, die die Ursachen der hohen Intersubjektvariabilität, mittels Identifikation von Untergruppen (z.B. Unterschiede auf Grund von genetischen Polymorphismen), berücksichtigen. Ein getrennter Vergleich dieser Probanden würde zu einer präziseren Einschätzung betreffend dieser Fragestellung führen und schließlich zu einem besseren Verständnis des Einflusses von Steroidhormonen auf die serotonerge Neurotransmission beitragen.

CONTENTS

1. INTRODUCTION

The serotonergic system is involved in the regulation of mood and affective states, appetite, sexual behavior and sleep and plays also a role in memory and learning. Dysfunctions in serotonergic neurotransmission are considered to be linked to manifestations of various psychiatric disorders like depression (Owens and Nemeroff, 1998, Drevets et al., 1999, Meltzer et al., 2004, Parsey et al., 2006, Drevets et al., 2007, Hirvonen et al., 2008, Parsey et al., 2010), schizophrenia (Burnet et al., 1997, Kasper et al., 1999, Stockmeier, 2003), or anxiety disorders (Kasper, 2001, Neumeister et al., 2004, Lanzenberger et al., 2007) and also affective states like fear or aggression (Baldwin and Rudge, 1995, Witte et al., 2009). In most studies of these disorders a decrease of 5-HT1A binding potential has been shown.

Due to the fact that suffering from major depression and anxiety disorders is two times more prevalent in women than in men (Alonso et al., 2004) it has been assumed that this could be a result of hormonal caused differences in the serotonergic system. Some of the serotonin receptors, like the main inhibitory 5-HT_{1A} and the excitatory 5-HT_{2A} receptor or the serotonin transporter, have been reported to be modulated by steroid hormones (Moses et al., 2000, Bethea et al., 2002b, Stockmeier, 2003, Lanzenberger et al., 2005, Lanzenberger et al., 2007, Lanzenberger et al., 2010, Moser et al., 2010).

Several studies on rodents or non-human primates have been conducted revealing influence of sex hormones on serotonergic transmission (Pecins-Thompson and Bethea, 1999, Lu and Bethea, 2002, Le Saux and Di Paolo, 2005). Also post mortem studies delivered insight into their effects, but only a few PET studies have been conducted regarding the interaction between gonadal hormones and the serotonergic system in vivo in the human brain.

Moses et al. investigated the influence of estradiol and progesterone administration on the 5-HT_{2A} receptor distribution in postmenopausal women which increased the binding potential in widespread areas of the cerebral cortex (Moses et al., 2000). These findings were consistent with those in a second study, performed three years later (Moses-Kolko et al., 2003). Kugaya et al. could confirm those results for estrogen, but only for the right prefrontal and the anterior cingulate cortex (Kugaya et al., 2003).

Jovanovic et al. examined the 5-HT_{1A} receptor binding potential in women with premenstrual dysphoric disorder compared with asymptomatic controls at two different phases of the menstrual

cycle by using PET and the selective radioligand [carbonyl-^{11}C]WAY100635 (Jovanovic et al., 2006). In 5 healthy controls the 5-HT_{1A} receptor BP values in the dorsal raphe nuclei were lower in the follicular and higher in the luteal phase of the menstrual cycle, but this difference was not statistically significant. In 2009 the same group investigated 13 healthy women to explore the effects of the menstrual cycle phases on 5-HT_{1A} receptor and 5-HTT binding potentials. Again they showed that the binding potentials did not differ significantly between follicular and luteal phases (Jovanovic et al., 2009). However, these studies have to be interpreted with caution due to the small sample size and short observation periods.

2. OBJECTIVES

The analysis aims to depict if there is a difference in 5-HT_{1A} receptor binding potential in pre- and postmenopausal women.

For this purpose data of the 5-HT_{1A} receptor binding potential measured using positron emission tomography, as well as blood samples of premenopausal women that have been recruited for the study "In vivo imaging of 5-HT_{1A} receptors using PET in patients with anxiety and healthy controls" (EK 318/2002) and data of postmenopausal women recruited for the study "The influence of hormone replacement therapy on the cerebral serotonin-1A receptor distribution and mood in postmenopausal women" (EC 593/2007) were analyzed.

3. HYPOTHESIS

1. **The menopausal status of pre- and postmenopausal women will have a significant effect on the nondisplaceable binding potential of the 5-HT_{1A} receptor.**
2. **Sex hormone plasma levels will be significantly correlated with regional 5-HT_{1A} receptor binding potential in healthy women.**

4. BACKGROUND

4.1. THE SEROTONERGIC SYSTSTEM

Serotonin is known to play a central role in several cerebral functions and affective states such as mood, anxiety, cognition, eating behavior and stress response (Aghajanian, 2002).

5-hydroxytryptamine (5-HT) is a small indolealkylamine, identified initially because of its cardiovascular effects. First it was named enteramine due to the fact that it was discovered in enterochromaffin cells (Erspamer, 1940, Erspamer and Boretti, 1950, Erspamer and Asero, 1952, Dalgliesh et al., 1953). Rapport et al. isolated this substance from the serum of cattle, hence this substance was named "serotonin" afterwards (Rapport et al., 1948a, b). Later on, the existence of serotonin and its activity as a neurotransmitter in the central and peripheral nerve system could be shown (Twarog and Page, 1953, Amin et al., 1954, Brodie et al., 1955).

1-2% of the serotonin can be found in the brain tissue, but in a large part it is located in platelets, mast cells and enterochromaffin cells (Gershon et al., 1985, Matzel and Kolata, 2010).

4.1.1. Structure, synthesis and metabolism of serotonin

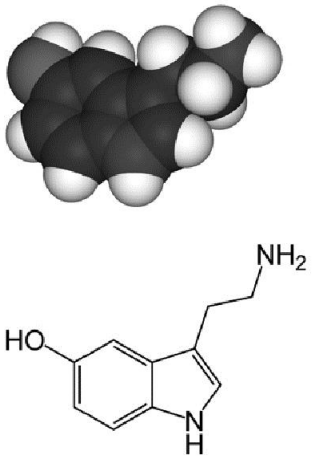

Figure 1 3D model and chemical structure of serotonin (adapted from Siegel et al. 2008)

Due to its hydroxyl group in the 5 position of the indole nucleus and nitrogen as a proton acceptor, at physiological pH 5-HT is a hydrophilic substance and therefore does not pass the lipophilic blood-brain barrier without an active-transport mechanism (Siegel GJ, 1999). Compared to platelets that accumulate 5-HT from plasma by means of an active transport mechanism, cells in brain tissue synthesize 5-HT in the cytoplasm of serotonergic neurons from the amino acid L-tryptophan which is being transported through blood-brain barrier by the same carrier that also serves other neutral amino acids, the large neutral amino acid transporter LNAAT (Narr et al., 2007).

L-tryptophan-5-monooxygenase, also termed tryptophan hydroxylase (TPH), performs the first step of serotonin synthesis by converting tryptophan (TRP) to 5-hydroxytryptophan (5-HTP). Two forms of tryptophan hydroxylase are known. Tryptophan hydroxylase 2 (TPH2) seems to appear only in 5-HT synthesizing neurons in the brain tissue and is the predominant isoform in the central nervous system (Duncan et al., 2000, Walther and Bader, 2003, Jacobsen et al., 2011), whilst TPH1 can be found in peripheral tissues and in the pineal gland (Gutknecht et al., 2009). The next step is conducted by L-amino acid decarboxylase (AADC) which is also present in catecholaminergic neurons and involved in dopamine synthesis. It decarboxylates 5-HTP to serotonin.

The first step appears to be the rate-limiting. Inhibition of this reaction by parachlorphenylalanin (PCPA) results in a marked depletion of the 5-HT levels in the brain while in general 5-HTP is only found in trace amounts in the brain, assumedly because it is decarboxylated about as rapidly as it is formed. Figure 2 shows the synthesis and metabolism of serotonin.

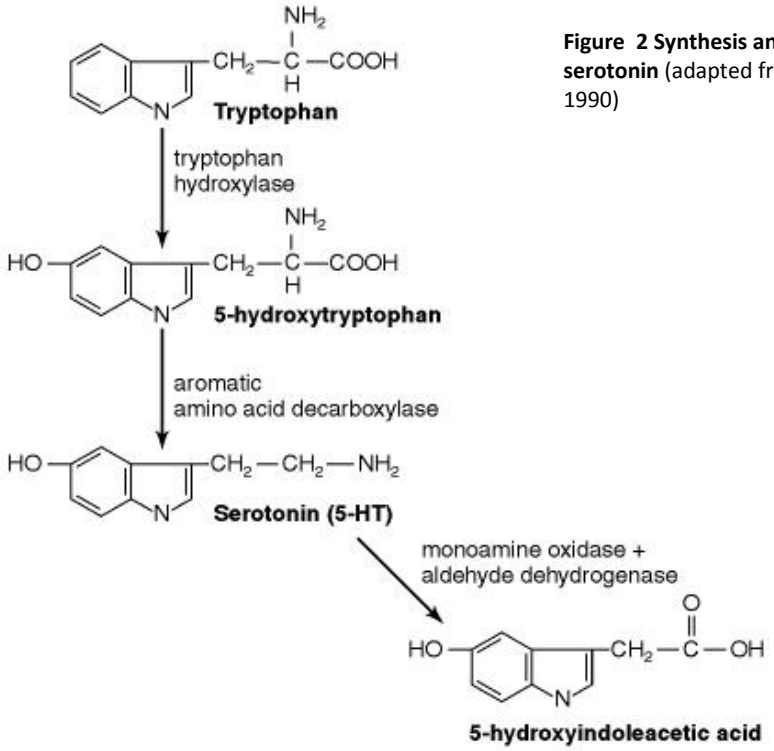

Figure 2 Synthesis and degradation of serotonin (adapted from Siegel et al. 1990)

To protect the fresh synthesized serotonin from degradation, 5-HT binding proteins (SBP) attach with high affinity to this indolealkylamin. The vesicular monoamine transporter proteins VMAT1 and VMAT2 (which is primarily expressed in the CNS) use the electrochemical gradient of H^+-ATPase to drive the transport of serotonin into storage vesicles. Recently, a vesicular-filling synergy of VMAT2 and VGLUT3 in serotonergic neurons lacking SERT was reported, which could also play a role as a regulatory mechanism in serotonin neurotransmission (Amilhon et al., 2010). In response to an action potential, the neurotransmitter serotonin can be released by exocytosis into the synaptic cleft. This process is accompanied by a quick influx of Ca^{2+}. The vesicle membrane fuses with the presynaptical plasma membrane of the nerve terminal resulting in discharge of the vesicle content.

The released serotonin either binds to pre- and postsynaptic serotonin receptors or exerts its influence by means of non-synaptic neurotransmission, also termed as diffuse or volume transmission (Descarries et al., 1975, Descarries and Mechawar, 2000, Agnati et al., 2010). The serotonin transporter (SERT) disrupts the effect of serotonin by reuptake of the released 5-HT into

the presynaptic neuron. There it can be either repackaged into storage vesicles or degraded by means of MAOA into 5-hydroxyindole acetic acid (5-HIAA). In the pituitary gland serotonin is metabolized to melatonin. The primary degradation product of serotonin, 5-HIAA, can be measured in the lumbar cerebrospinal fluid or as a urinary metabolite, indicating either the turnover in brain tissue or amount of serotonin in the human body in general. Figure 3 shows the transportation of tryptophan through the blood-brain barrier, as well as the synthesis, reuptake and degradation of serotonin.

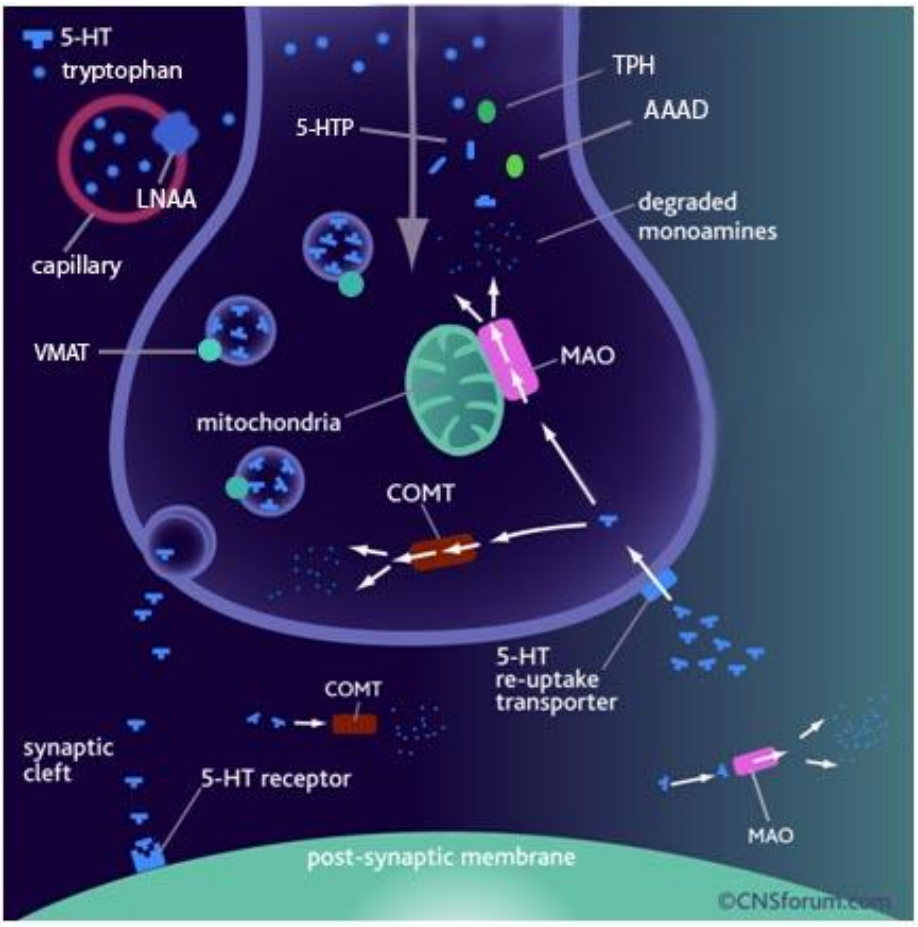

Figure 3 Synthesis and metabolism of serotonin; this is a schematic, the SERT molecules are not expressed at the synapses (adapted and modified from www.cnsforum.com, Lundberg Institute)

4.1.2. Pathways of the serotonergic system in the human brain

The 5HT neurons share a firing pattern which is high during the day and very low at night during sleep. Therefore the highly regular pacemaker activity of serotonin neurons is considered to be dependent on the waking state (Jacobs and Azmitia, 1992, Azmitia, 1999, 2007).

The organization of the serotonin neurons is more evolved in the primate, where highly myelinated fibers are common compared to the rodents where highly myelinated axons are rare. In rodents thin, highly branched, and unmyelinated axons are the predominant form (Azmitia, 2007).

Several authors describe the distribution of 5-HT cell bodies in the human brain as restricted to the brain stem (Baker et al., 1991a, Baker et al., 1991b, Hornung, 2003). These are approximately 350 000 cells and a majority of them is concentrated along the midline in the raphe nuclei.

5-HT neurons from the raphe nuclei make connections innervating the entire brain and spinal cord (Azmitia, 2007).

According to cell body localization and their projections, 5-HT neurons can be separated into two groups (Charnay and Leger, 2010):

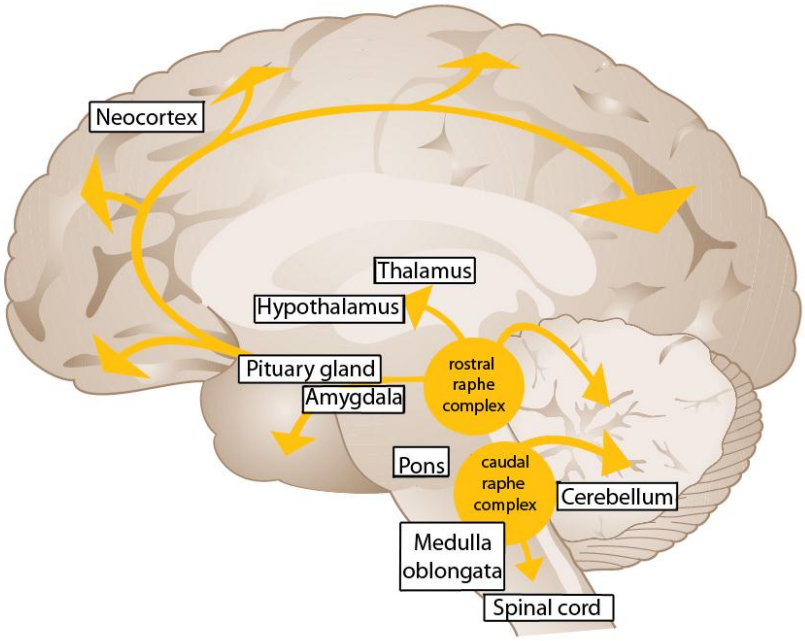

Figure 4 Projections of the serotonergic system in human brain, sagittal section (adapted and modified from Kriegebaum et al. 2010)

Rostral (or superior) group:

- located in the mesencephalic and rostral pons
- sending axons to the forebrain (cerebral cortex and limbic system)
- approximately 85% of the 5-HT neurons
- composed of neurons located in four nuclei:
    - interpeduncular, caudal linear, dorsal and median raphe nuclei

- additional area is the caudal mesencephalic and rostral pontine reticular formation

Caudal group:

- located in rostral pons and medulla oblongata
- sending axons to brainstem and spinal cord
- approximately 15% of the 5-HT neurons
- composed in three raphe nuclei:
  - raphe magnus, raphe obscurus, raphe pallidus
- additional area is ventral medullary reticular formation

A detailed Figure showing the raphe nuclei in a sagittal section can be found in the annex (Annex 13.1, Fig. 5).

4.1.3.Serotonergic receptor subtypes

The serotonin receptors were described first in the guinea-pig ileum during the 1950s. They were called M and D receptors due to their sensitivity to morphine or dibenzyline (Gaddum and Picarelli, 1957). Later on electrophysiological, pharmacological and binding experiments followed.

To date more than 15 5-HT receptors, grouped into seven families (5-HT_{1-7}), were discovered by means of various cloning strategies. They mainly appear as postsynaptic receptors. To date the 5-HT_{1A} and 5-HT_{1B} receptors were identified as presynaptic autoreceptors modulating serotonin release in a negative feedback manner.

The 5-HT receptors are G-protein coupled (i.e. metabotropic) receptors activating various signaling pathways (Fig. 6). 5-HT_3 receptors (Barnes et al., 2009) are an exception and belong to ionotropic receptors. There is evidence that the receptor density (i.e. the number of receptors expressed on the surface of neurons) vary due to sustained stimulation by agonists or endogenous 5-HT resulting in attenuated receptor responsiveness (desensitization), intracellular sequestration (internalization) and recycling back to the membrane (Idkowiak-Baldys et al., 2009). Also the crosstalk with other receptors, homodimerisation, heterodimerisation, as well as a variety of proteins including for example ß-arrestins, influence the activity of 5-HT receptors, but are described elsewhere (for further details see Gonzalez-Maeso et al. 2007, Allen et al. 2008).

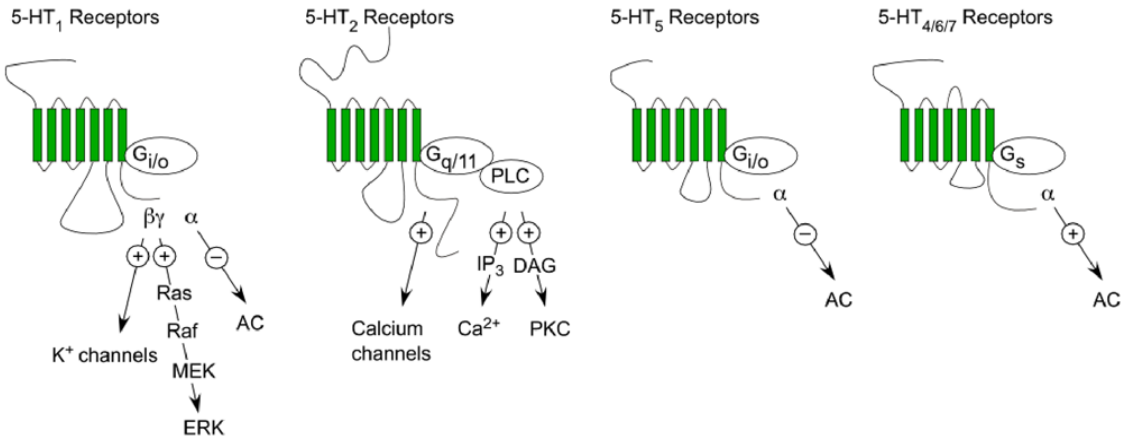

Figure 6 G-protein coupled 5-HT receptors - Signaling pathways; **AC** – adenylyl cyclase; **DAG** – diacylglycerol; **ERK** – extracellular signal regulated kinase; **IP3** – inositol triphosphat; **MEK** – mitogen and extracellular signal regulated kinase; **PKC** – protein kinase C (taken from Roth et al. 2006)

4.2. THE 5-HT$_{1A}$ RECEPTOR

4.2.1. Structure and function of the 5-HT$_{1A}$ receptor

The 5-HT$_1$ receptors are divided into five subfamilies, termed 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ receptors. The former 5-HT$_{1C}$ receptor has been reclassified as the 5-HT$_{2C}$ receptor due to similarities to other 5-HT$_2$ receptors.

The 5-HT$_{1A}$ receptor, as the main inhibitory serotonergic receptor, is involved in diverse processes such as neuroendocrine regulation, vasoreactive headaches, thermoregulation, sexual behavior, food intake and appetite, memory, depression, aggression, anxiety, and immune function (Roth, 2006). Activation of 5-HT$_{1A}$ receptors reduces cell firing in hippocampus (Kasamo et al., 2001), forebrain (Ashby et al., 1994) and raphe nuclei (Haddjeri et al., 2004).

This inhibitory receptor is found on both, the presynaptic as well as the postsynaptic neuron. 5-HT$_{1A}$ receptors are G-protein coupled (GPCR) and consist of seven hydrophobic transmembrane domains connected by three intracellular and three extracellular loops with an amino terminus orientated toward the extracellular space and a carbonyl terminus oriented toward the cytoplasm (Fig. 7). They have been characterized biochemically and electrophysiologically as $G_{i/O}$ receptors since they are sensitive to inactivation through pertussis toxin (PTX).

These GPCRs couple to multiple secondary signaling pathways, which vary depending on for example location or cell type. In the brain tissue there are mainly two relevant effector systems:

- inhibition of adenylyl cyclase (AC) and
- the opening of K^+ channels (GIRK)

The effector of the $G_{\alpha i/o}$ pathway is the cyclic-adenosine monophosphate (cAMP) generating enzyme adenylyl cyclase (AC). $G_{\alpha i/o}$ inhibits AC and therefore prevents AC from producing cAMP.

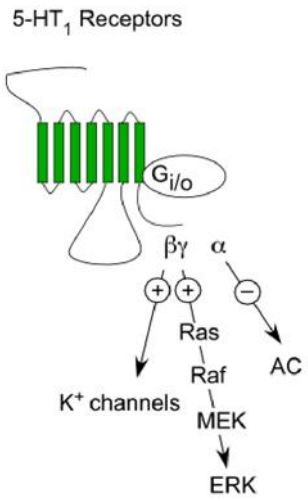

Figure 7 5-HT$_{1A}$ receptor – major signaling linkages

(taken from Roth et al. 2006)

The activation of the second effector system results in neuronal hyperpolarisation, thus inhibiting 5-HT cell firing and reducing serotonin release in the cell soma and in projection structures (Sinton and Fallon, 1988, Adell et al., 1993, Penington et al., 1993).

Both effector systems occur in terminal field areas of the serotonergic system such as hippocampal (De Vivo and Maayani, 1986), cortical and various other cultured neuronal cells (Siegel GJ, 1999), whereas in the dorsal raphe nucleus 5-HT$_{1A}$ receptors are coupled only to GIRKs, G protein-gated inwardly rectified K^+ channels (Innis and Aghajanian, 1987, Clarke et al., 1996).

Moreover there is evidence that 5-HT$_{1A}$ receptors activate extracellular signal regulated protein kinase (ERK) by means of ßγ-subunits (Cowen et al., 1996, Della Rocca et al., 1999, Adayev et al., 2003, Crane et al., 2007). Some authors report that 5-HT also inhibits calcium current through G protein coupling to voltage-dependent calcium channels in the dorsal raphe nucleus and the hypothalamus (Penington and Kelly, 1990, Penington et al., 1991, Rhee et al., 1996). However, some pathways are not mentioned here and there are still definitely more to discover (Cowen et al., 1996, Mannoury la Cour et al., 2006, Gu et al., 2007).

4.2.2. Distribution of the 5-HT$_{1A}$ receptor in the human brain

A clear distinction should be drawn between presynaptic and postsynaptic receptors due to their differences in localization and final function.

The raphe nuclei containing serotonergic neurons demonstrate high presynaptic 5-HT$_{1A}$ autoreceptor densities (Siegel GJ, 1999, Hornung, 2003, Charnay and Leger, 2010) while postsynaptic heteroreceptors are known to be located on glutamatergic and GABAergic neurons in the prefrontal cortex in humans (de Almeida and Mengod, 2008) and rodents or non-human primates (Santana et al., 2004, Palchaudhuri and Flugge, 2005, de Almeida and Mengod, 2008, Andrade, 2011).

Post-mortem human studies investigating the regional distribution of postsynaptic 5-HT$_{1A}$ heteroreceptors are consistent with studies in vivo showing very dense binding in hippocampus as well as other limbic structures and insula, a slightly lower binding in neocortex, in particular frontal, temporal and limbic cortical structures, low binding in amygdala, septum and claustrum and negligible binding in basal ganglia (nucleus caudatus, putamen), brainstem (except raphe nuclei), pallidum, thalamus and cerebellum (Hall et al., 1997, Andree et al., 2002, Moller et al., 2007, Moller et al., 2009).

Sustained administration of 5-HT$_{1A}$ receptor agonists or an increased serotonin availability induced by serotonin reuptake inhibitors (SSRI) were shown to lead to internalization of the 5-HT$_{1A}$ autoreceptors in the serotonin containing neurons, but not of hippocampal postsynaptic 5-HT$_{1A}$ receptors (Haddjeri et al., 1999, Riad et al., 2004, Zimmer et al., 2004). This different desensitization pattern is thought to add to the antidepressant or anxiolytic effects of various pharmaceuticals (Haddjeri et al., 1999, Blier and Ward, 2003).

Fig. 14 shows a schematic, illustrating pre- and postsynaptic 5-HT$_{1A}$ receptors, while Fig. 8 provides PET images in a single subject of the current study of both, presynaptic and postsynaptic receptors.

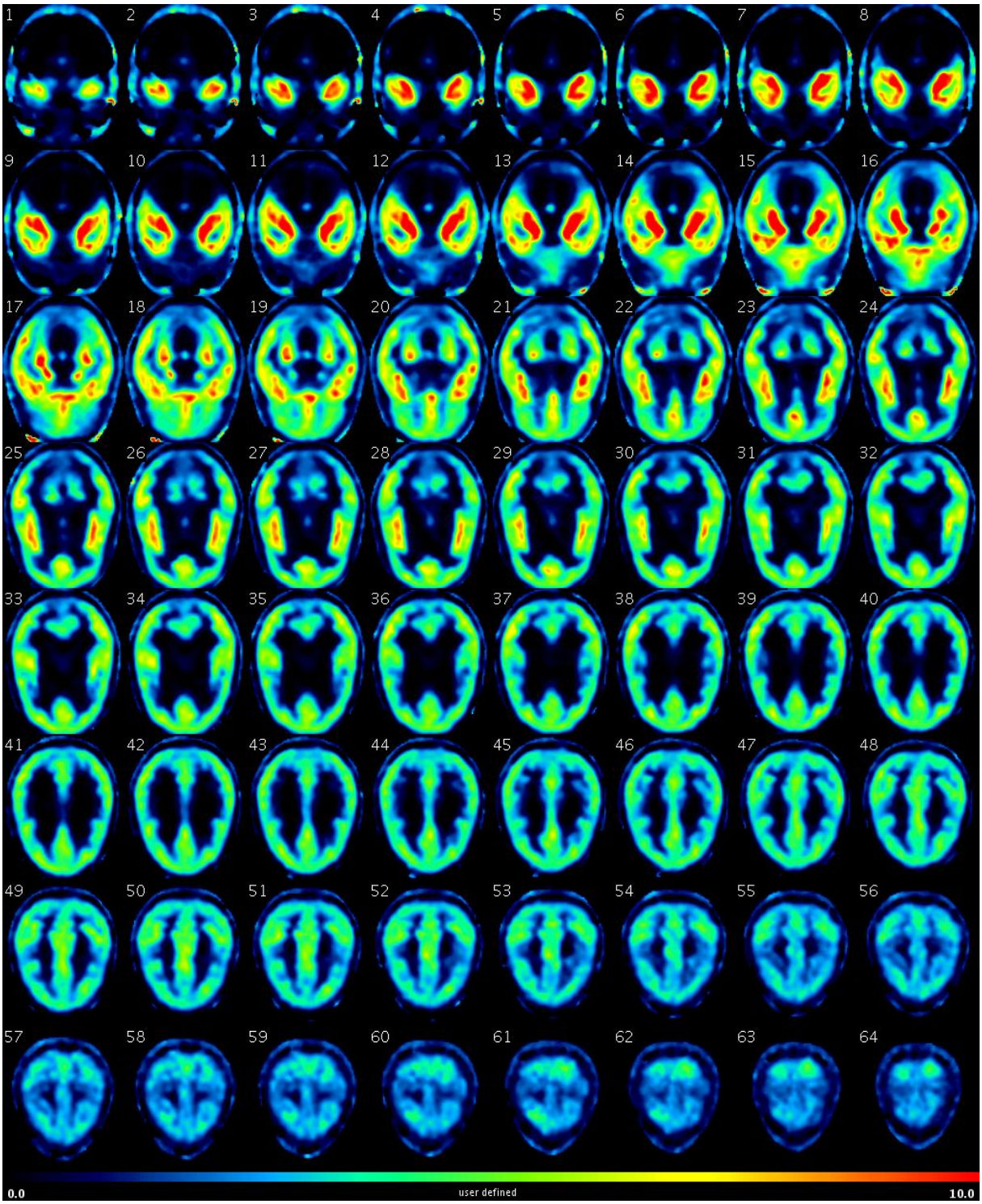

Figure 8 5-HT$_{1A}$ receptor binding potential in a single subject, transaxial slices; red – high 5-HT$_{1A}$ RBP, **blue** – low 5-HT$_{1A}$ RBP

4.3. THE STEROID HORMONES PROGESTERONE, ESTRADIOL AND TESTOSTERONE

Cholesterol is the precursor of the steroid hormones progestagens, glucocorticoids, mineralocorticoids, androgens, and estrogens (Fig. 9). Nuclear receptors for steroid hormones are members of a superfamily of ligand-dependent transcription factors. Considering the focus of the current study this chapter will give a review on progesterone, estradiol as well as testosterone and their respective nuclear receptors. For a review on membrane steroidal receptors the reader is referred to a paper published by Ramirez et al. (Ramirez and Zheng, 1996).

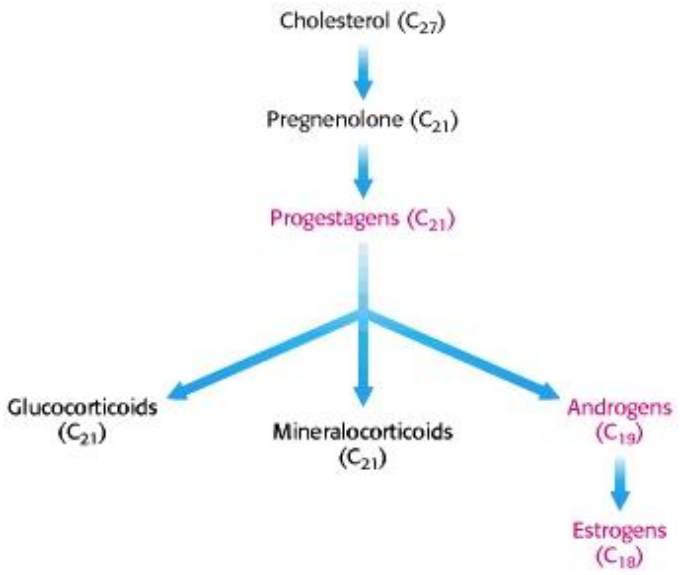

Figure 9 Biosynthetic relations between progestagens, androgens and estrogens (taken from Berg et al. 2002)

4.3.1. Structure, synthesis and effects of progesterone

Progesterone is synthesized in the ovary, testis, as well as the adrenal gland and during pregnancy also in the placenta. Progestins enhance differentiation and oppose the cell proliferation effects of estrogen, are involved in processes like ovulation, implantation, maintenance of pregnancy, mammary gland development and beside its hormonal effects progesterone serves as a precursor in the synthesis of other steroids like estrogens and androgens.

In the central nervous system progesterone appears to have depressant and hypnotic effects (Lu et al., 2006). It acts through regulation of gene expression, modulation of neurotransmitter systems as well as activation of signaling cascades and is involved in regulation of memory and neuronal

excitability as well as neurogenesis. Data from several sources indicate also neuroprotective actions (Brinton et al., 2008).

As shown in Fig. 10 progesterone is synthesized from pregnenolone which itself is synthesized from the steroid hormone precursor cholesterol.

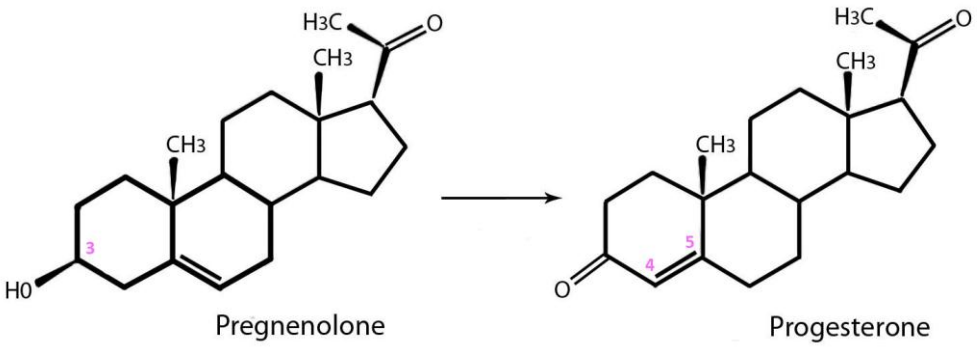

Figure 10 Progesterone synthesis. The 3-hydroxyl group of pregnenolone is oxidized to a 3-keto group. The Δ^5 double bond is isomerized to a Δ^4 double bond (Taken from Berg et al. 2002)

4.3.2.The progesterone receptor

The progesterone receptor (PR) was detected in the female reproductive tract, mammary gland and the brain tissue (Mangal et al., 1997). In humans PRs were traced in the hypothalamus, frontal cortex, hippocampus and amygdala (Sarrieau et al., 1986) by means of autoradiography, while in rodents the highest expression of PRs was found in the hypothalamus, medial preoptic area, cingulate cortex, the hippocampus and the medial amygdala (Levine et al., 2001).

Two isoforms of the progesterone receptor were identified, called PRA and PRB, which are transcribed from different promoters in the same gene. PRB seems to be a much stronger transcription activator than PRA. (Giangrande and McDonnell, 1999, Richer et al., 2002, Leonhardt et al., 2003). As shown in Fig. 11, binding of progesterone to a progesterone receptor is followed by homodimerisation and binding to DNA by means of progesterone response element resulting in increased or decreased target gene transcription. For detailed information regarding the progesterone receptor the reader is referred to an extensive review conducted by Brinton et al. (Brinton et al., 2008).

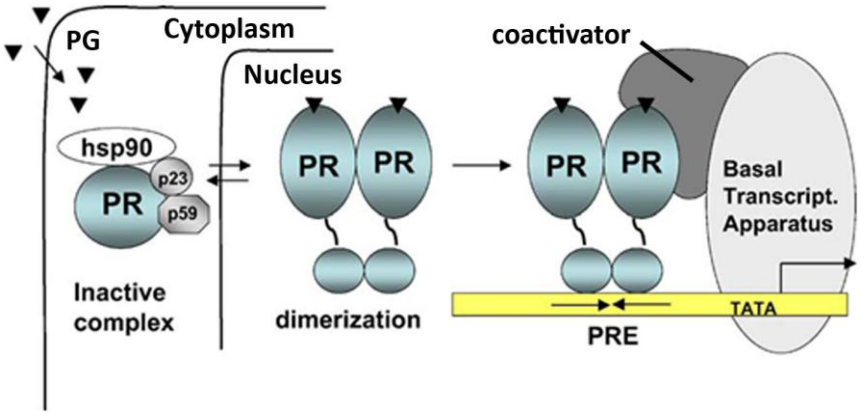

Figure 11 Activation of PR by progesterone binding; PG – progesterone, PR – progesterone receptor, PRE – progesterone response element (taken from Leonhardt et al. 2003)

4.3.3. Structure, synthesis and effects of estrogen

Three types of estrogen are produced in the ovaries:

- estrone, E1

- 17 beta-estradiol, E2

- estriol, E3

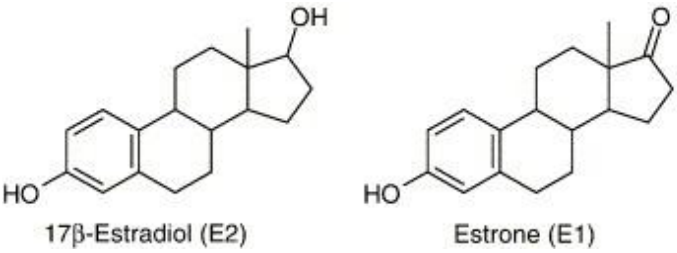

Figure 12 Chemical structure 17 beta-estradiol E2 and estrone E1 (taken from Czajka et al. 2006)

The predominant estrogen in premenopausal women is estradiol. Estrone is also produced in the fat tissue and therefore a common estrogen in postmenopausal women due to the fact that the ovarian steroid production ceases in this phase of life. E2 is converted to E1 and vice versa. Both steroidal hormones are equivalent in their level of estrogenic activity. Their chemical structure is shown in Figure 12. Estriol, E3, is made in the placenta during pregnancy and has the weakest effects of the three estrogens (Berek and Novak, 2007).

Synthesis of estrogens

Estradiol biosynthesis in human brain tissue is catalyzed by the enzyme aromatase (Fig. 13) which is expressed in cerebral cortex, basal forebrain, hippocampus, thalamus, cerebellum, brainstem hypothalamus, amygdala and preoptic/septal areas (Azcoitia et al., 2011).

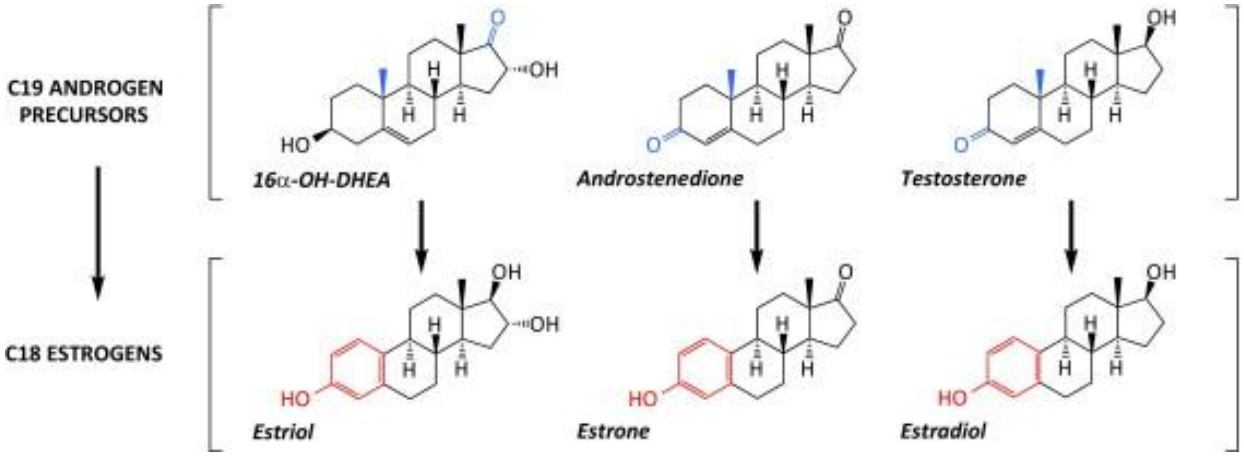

Figure 13 Aromatization of precursor androgens by aromatase enzyme. Three possible aromatase catalyzed reactions are depicted. (taken from Azcoitia et al. 2011)

Effects of estrogens

The growth, differentiation and function of tissues of the female reproductive system as well as non-reproductive tissues such as the cardiovascular system, bones and the brain are influenced by estrogens. Estradiol prevents neuronal cell death, promotes the formation of synapses and modulates learning and memory (Wilson et al., 2011).

Estrogens increase tryptophan hydroxylase (TPH) 1 and 2 expression in rodent and primate raphe nuclei (Bethea et al., 2000, Gundlah et al., 2005, Sanchez et al., 2005, Donner and Handa, 2009, Osterlund, 2010) and downregulate MAO mRNA in non-human primates and rats (Ortega-Corona et al., 1994, Gundlah et al., 2002). This downregulation can be reversed by progesterone (Luine and Rhodes, 1983). The 5-HT transporter also appeared to be downregulated in animals (Pecins-Thompson and Bethea, 1999) but a more recent finding traced this effect only in female rats (Benmansour et al., 2009). All this actions result in increased 5HT availability. It is assumed that this processes account for the serotonin system stimulating effects. Fig. 14 summarizes these effects in an example of presynaptic 5HT neurons and postsynaptic target neurons, but it has to be noted that previous research is still inconclusive and the effects differ based on region and involved cell type. Also differences between species could be traced and therefore, as long as research data for humans

are scarce, these findings cannot be generalized. A detailed review on effects of steroid hormones on 5-HT_{1A} BP can be found in the discussion of the current study.

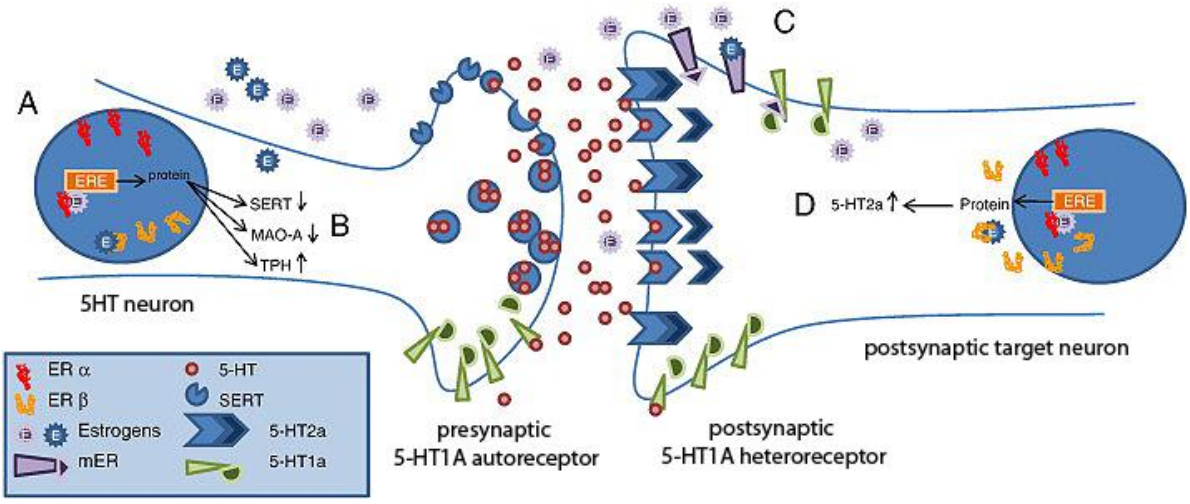

Figure 14 Exemplary illustration of estrogen effects on serotonergic neurotransmission

(A) Estrogens bind to nuclear ERs. Together with EREs the ERs act as transcription factors altering the expression of a number of genes related to serotonergic neurotransmission. **(B)** The resulting alterations in protein expression i.e. SERT, TPH and MAO-A proteins lead to a changed 5-HT concentration. Increased 5HT levels subsequently desensize 5-HT_{1A} receptors. **(C)** 5-HT_{1A} receptor function is modified by mERs trough uncoupling of G-proteins resulting in deactivation of 5-HT_{1A} **(D)** 5-HT_{2A} receptors have been shown to be increased after estrogen administration. **ER** – estrogen receptor, **ERE** - estrogen response element, **MAO-A** - monoamine oxidase A, **SERT** – serotonin reuptake transporter, **TPH** - tryptophan hydroxylase (taken and modified from Hildebrandt et al. 2010)

In terms of other transmitter systems estrogen treatment reduces tyrosine hydroxylase activity and therefore catecholamine synthesis but increases choline acetyltransferase and acetylcholinesterase activity (Luine and McEwen, 1983). The latter effects may also be a part of the antidepressant effect of estrogen (Rossmanith and Scherbaum, 1992).

Effects of estrogens on 5-HT_{1A} mRNA expression, the 5-HT_{1A} receptor distribution and 5-HT_{1A} receptor function

In **rodents** Lakoski et al. demonstrated that estrogen administration reduces the ability of the 5-HT_{1A} agonist [3H]8-OH-DPAT to decrease firing at dorsal raphe neurons, indirectly indicating reduced **[3H]8-OH-DPAT binding sites** of this **autoreceptors** (Lakoski, 1988). Another study conducted by Birzniece et al. revealed a decrease of 13% in the **5-HT_{1A} mRNA expression** in the ventrolateral part

of the DRN of rats after two weeks of estrogen and progesterone treatment, while no change was found in the median raphe nucleus (Birzniece et al., 2001).

Ovarectomy and hence hormone withdrawal provoked an increase of **BP** and **receptor function** in the dorsal raphe of rats which could be corrected by E_2 administration (Le Saux and Di Paolo, 2005).

Pecins-Thompson and Bethea et al. investigated the influence of estrogen, as well as estrogen and progesterone administration in **non-human primates** and demonstrated similar findings to studies in rodents with a decrease of 5-HT_{1A} receptors and 5-HT_{1A} mRNA in the dorsal raphe nucleus (Bethea et al., 1998, Pecins-Thompson, 1998, Bethea et al., 2002a). The addition of progesterone during 28 days of estrogen treatment further reduced 5-HT_{1A} gene expression (Pecins-Thompson and Bethea, 1999). A recent study in rhesus macaques revealed that the suppressive effect of estrogens on 5-HT_{1A} protein levels in DRN seen after one month of estrogen administration diminished after five months of treatment with estrogen. An additional administration of progesterone counteracted this effect resulting in significantly decreased 5-HT_{1A} receptor protein levels even after five months of hormone replacement (Henderson and Bethea, 2008). However, there is also some evidence that estrogen replacement does not change BP or mRNA levels in DRN of rats (Landry and Di Paolo, 2003).

Surveys regarding **postsynaptic** receptors such as these conducted by Mize, Alper et al. (2000, 2000) showed that estrogen decreases **5-HT_{1A} function** (e.g. acute E_2 administration reduced agonist-stimulated [35S]GTPgammaS binding by ca. 25% in the hippocampus, cortex and amygdala of rats *in vivo)* . Several similar studies by the same group, with different focus or design, indicated this rapid effect of estrogen on phosphorylation of the 5-HT_{1A} receptor resulting in uncoupling from the G protein (Mize et al., 2001, Mize and Alper, 2002, Mize et al., 2003).

On mRNA level Österlund et al. reported a reduction of **5-HT_{1A} mRNA** after short-term administration of E_2 in medial amygdala, piriform cortex and perirhinal cortex, while no change appeared in hippocampus and retrosplenial cortex in the brain tissue of rats, showing a region specific alteration in limbic related areas (Osterlund et al., 1998). In a second study they investigated 5-HT_{1A} mRNA alterations after long-term administration of E_2 and no change in 5-HT_{1A} mRNA levels was shown. Österlund suggests that the acute alteration diminishes with chronic treatment (Osterlund et al., 2000).

In contrast, two weeks of estradiol application decreased **5-HT_{1A} receptors** in amygdala, hippocampus, perirhinal and motor cortex of ovarectomized rats. The influence of estradiol seems to be region specific also in case of postsynaptic 5-HT_{1A} receptors, since no alteration appeared in other regions investigated, like piriform and retrosplenial cortex (Osterlund et al., 2000).

A constant receptor density in hypothalamus-preoptic area and hippocampus of rodents was reported despite E_2 administration (Clarke and Maayani, 1990, Jackson and Etgen, 2001). The anterior prefrontal and cingulate cortex 5-HT_{1A} BPs remained unaffected by estrogen fluctuation due to the fact that neither ovarectomy nor estrogen administration altered the [3H]8-OH-DPAT-specific binding of this postsynaptic receptors in rodents. Data from the same investigation revealed that ovarectomy had no effect on hippocampal 5-HT_{1A} receptor binding and estrogens decreased BP only in a subregion of the CA3 (Le Saux and Di Paolo, 2005). Some other studies also reported missing alterations of BP in rats after estrogen replacement therapy. Landry et al. did not find changes in the subregions of the hippocampal formation, prefrontal cortex or cingulate cortex (Landry and Di Paolo, 2003) and Flügge et al. reported unchanged BP in medial preoptic area, stria terminalis, lateral septum, cingulate cortex, amygdala, hippocampal region and occipital cortex. In the ventromedial hypothalamic nucleus they even found an increase in BP during oestrus compared to dioestrus cycle phase of rats (Flugge et al., 1999).

4.3.4. The estrogen receptor

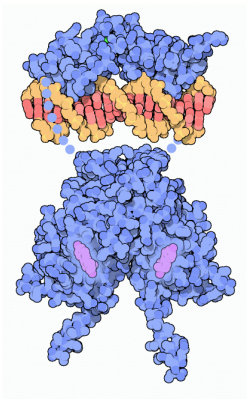

Figure 15 Estrogen receptor (taken from RCSB Protein Data Bank www.pdb.org, PDB ID 1hcq)

The estrogen receptor (ER) belongs to the nuclear receptor superfamily for steroid and thyroid hormones, vitamin D, retinoids and prostanoids (Parl, 2000). The two known receptors ERalpha and ERbeta were cloned 1986 (Green et al., 1986, Greene et al., 1986) and 1996 (Kuiper et al., 1996), respectively. They both act as dimers to regulate transcriptional activation. There is 96% amino acid identity between the two receptors in the DNA-binding domain, whereas the ligand-binding domain is homologous in only 5% (Weihua et al., 2003, Dahlman-Wright et al., 2006). In the human and rodent brain ERs can be found in limbic-related areas such as limbic cortices, amygdala, hippocampus and hypothalamus (Osterlund, 2010).

4.3.5. Structure, synthesis and effects of testosterone

Testosterone is synthesized from the steroid precursor cholesterol. Fig. 16 shows the involved enzymes and relation to other steroidal hormones.

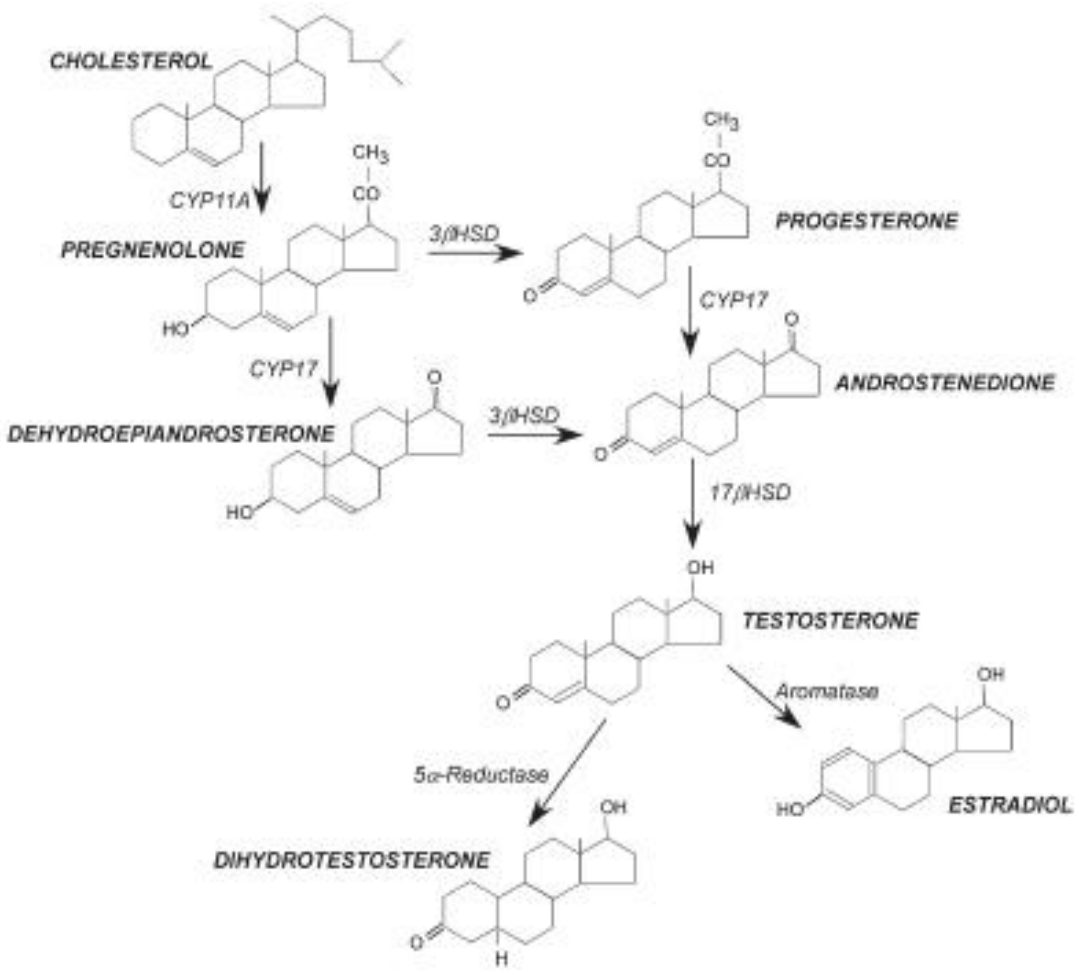

Figure 16 Testosterone synthesis pathway - 3βHSD - 3β hydroxysteroid dehydrogenase, **17βHSD** - 17β-hydroxysteroid dehydrogenase, **CYP17** - Cytochrome P450 (adapted and modified from Handa et al. 2008)

Testosterone can either bind directly to the androgen receptor, is locally metabolized in neural tissue to E2 and DHT by the enzymes aromatase and 5α-reductase, respectively, or is converted into an androgenic metabolic form that binds to neither the AR nor the ER (Kawata, 1995, Lephart et al., 2001).

Beside its influence of intermale aggressive behavior (Simon et al., 1998) testosterone and its metabolites showed rewarding properties (Frye et al., 2002), as well as anti-anxiety and analgesic effects (Edinger and Frye, 2005) in rodents and a negative feedback regulation of gonadotropin secretion (Urban et al., 1991). It was proven that they have an impact on sex differences in the morphology of particular brain regions, on size and number of neurons and glial cells or the number of synapses (Panzica et al., 1995, Cooke et al., 1998, Fernandez-Guasti et al., 2000, Melcangi et al., 2011).

4.3.6. The androgen receptor

The research to date regarding the distribution of androgen receptors has tended to focus on rodents, non-human primates or other species, whilst human studies are scarce. ARs in the human brain tissue have been traced in the hypothalamus (Fernandez-Guasti et al., 2000) and temporal cortex (Sarrieau et al., 1990, Puy et al., 1995). A preliminary study conducted by Bezdickova et al. showed nuclear expression of ARs within the hippocampus, precentral gyrus and the mamillary body in males but not in the brain tissue of the female subject. Cytoplasmic ARs were more widespread showing positivity in males and females in parts of the archi-cortex (Bezdickova et al., 2007).

In rats androgen receptors could be found in the hypothalamus, preoptic area, cortical structures and amygdala (Naess, 1976, Menard and Harlan, 1993, Lynch and Story, 2000, Fernandez-Guasti et al., 2003). Sheng et al. identified ARs in the DRN of male rats, but could not trace them in females (Sheng et al., 2004).

In addition to effects via gene transcription, androgen receptors activate membrane and cytoplasmic signaling pathways (DonCarlos et al., 2006).

4.4. PREMENOPAUSE

4.4.1. Definition

The WHO defines premenopause as the whole of the reproductive period prior to menopause (WHO research group, no authors listed 1996).

Approximately two third of adult women have menstrual cycles that last from 21 to 35 days, therefore many women experience anovulatory or irregularly timed cycles during reproductive life. On average the menstrual bleeding lasts 2 to 6 days with a blood loss of 20-60 mL (Berek and Novak, 2007).

The ovarian cycle can be divided into follicular and luteal phases:

1) **Follicular phase** - The average length ranges from 10 to 14 days. A single dominant follicle develops by means of hormonal feedback and is usually mature and prepared for ovulation at midcycle.

2) **Luteal phase** - The average length is 14 days and includes the time from ovulation to the onset of menses.

4.5. POSTMENOPAUSE

4.5.1. Definition

Menopause is not a central event, but primary ovarian failure, even though it is associated with changes in the hypothalamic and pituitary hormones. A depletion of ovarian follicles occurs and therefore the ovary is no longer able to respond to the pituitary hormones, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Ovarian estrogen and progesterone production ceases. Menopause is defined retrospectively as the time of the final menstrual period followed by 12 months of amenorrhea (Fig. 17).
Postmenopause is defined as the period following the final menses (Berek and Novak, 2007).

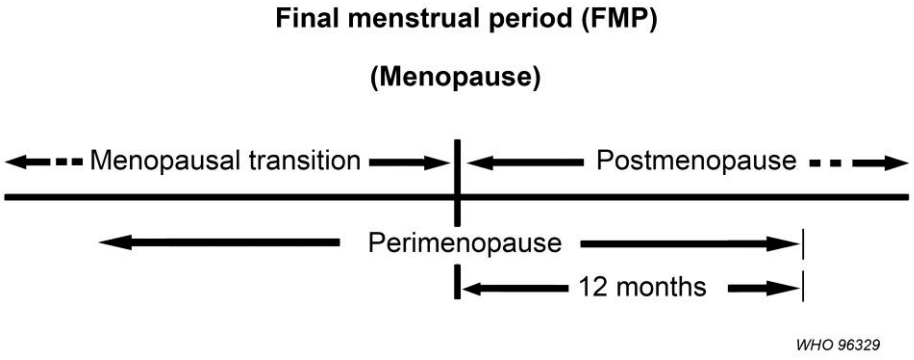

Figure 17 Different time periods surrounding the menopause
(adapted and modified from WHO research group, no authors listed 1996)

Most commonly noted complaints by perimenopausal woman include hot flushes, muscle and joint pain, weight gain, fatigue, headaches, irritability, low mood, and insomnia (Stuenkel, 1989), but only 35% of women seek medical treatment because of this symptoms (Backstrom, 1995).

4.6. POSITRON EMISSION TOMOGRAPHY

4.6.1. Physical principles and technology of PET imaging

Positron emission tomography (PET) is a radionuclide imaging procedure. It uses radiolabels and electron-positron annihilation reaction-induced gamma rays to locate incorporated radioligands which, like in case of 5-HT_{1A} receptors, bind to selected targets.

A radiotracer (also termed radiopharmaceutical or radioligand) consists of two components:

(1) a molecular structure (vector, vehicle, ligand) and
(2) a positron emitting radionuclide.

The molecular structure determines the distribution of the radiotracer within the organism due to pharmacokinetics and their pharmacodynamic properties. The radioactive nuclide is responsible for the signal which is detectable outside of the organism. Since radiotracers are chemically indistinguishable from their non-radioactive counterparts they serve as substitutes without altering biochemical processes (Wadsak and Mitterhauser, 2010)

The emitted signals resulting from processes mentioned above can be detected outside the subject's body and finally converted into an electronic representation or image of kinetic processes (regional blood flow, fatty acid or glucose metabolism). Also quantifications of molecules (receptors, transporters, antigens, enzymes and other proteins) are possible and can be visualized by this means in vivo.

The positron emitting isotopes are generated by means of a cyclotron and serve as a label on a specific tracer which gets incorporated by a subject for example through injection. The tracer disperses via blood stream and in case of [carbonyl-^{11}C]WAY100635 it penetrates the blood-brain barrier. The radionuclide in the tracer decays and the released positrons subsequently annihilate (Fig. 18) when converging with electrons after travelling a short distance of approximately 1 mm within the body (Cherry, 2006). This distance, also called positron range, leads to spatial uncertainty in the annihilation localization and is a limiting factor of the detection precision of the scanner (i.e. PET spatial resolution).

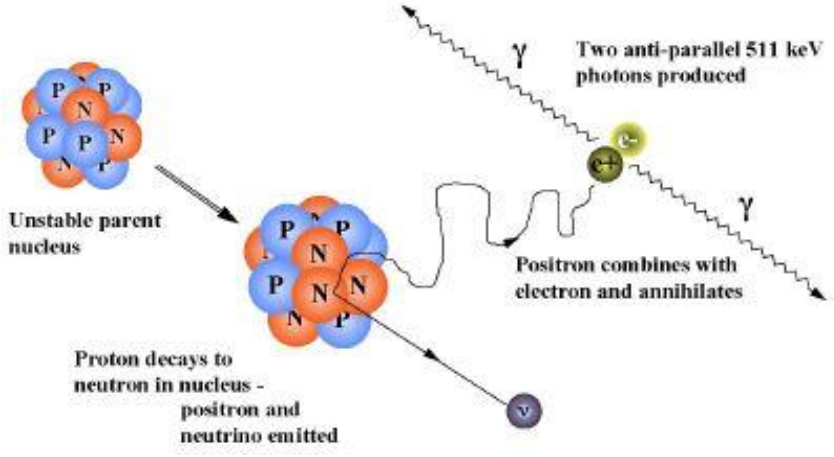

Figure 18 Positron emission and annihilation process (taken from Badawi 1998)

The emitted gamma rays get registered simultaneously by electronically linked detectors surrounding the subject. Those high sensitive scintillation detectors convert the gamma rays into visible light and the optically coupled photomultiplier tube (PMT) hence generates an electric signal for each event which is considered as "coincident" when occurring within a certain time window (Fig. 19). The imaginary line that joins the two opposite detectors that register the coincidence event is defined as Line of Response (Badawi, 1999).

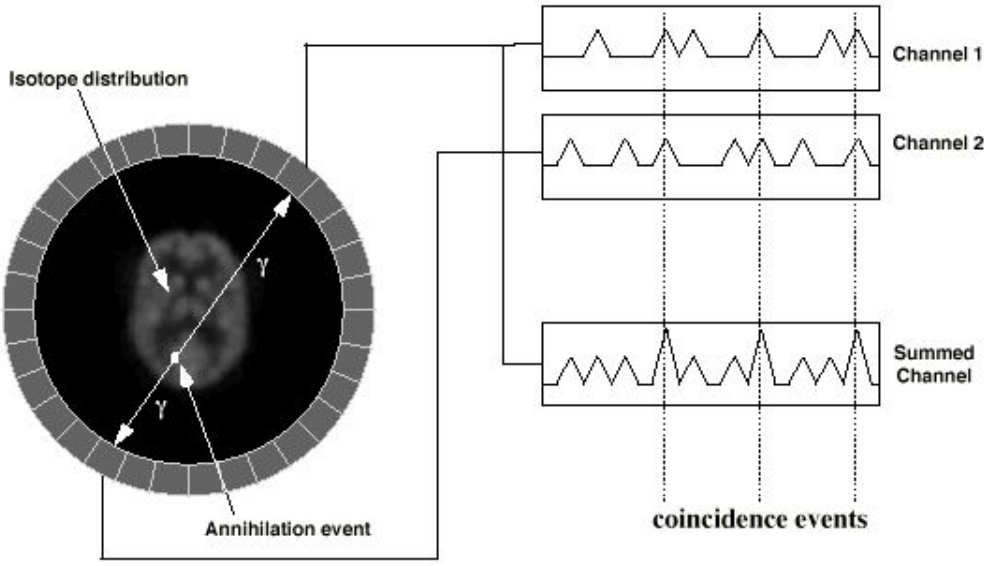

Figure 19 Coincidence detection in a PET camera (taken from Badawi 1998)

4.6.2. [Carbonyl-^{11}C]WAY100635

N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY100635) is a highly specific antagonist of the serotonin-1A receptor with a dissociation constant K_D of 0.2nM. After labeling with carbon-11 in its carbonyl position the new reversibly binding radioligand [carbonyl-^{11}C]WAY100635 emerged in 1996 (Pike et al., 1996). Its chemical structure is shown in Fig. 20. As an advantage to previous tracers targeting 5-HT_{1A} receptors its metabolite WAY100634 is non-radiolabeled and therefore [carbonyl-^{11}C]WAY100635 is the only source of radioactivity that can be traced during PET measurement. To date [carbonyl-^{11}C]WAY100635 is the most commonly used ligand for *in vivo* human studies for quantification of 5-HT_{1A} receptors.

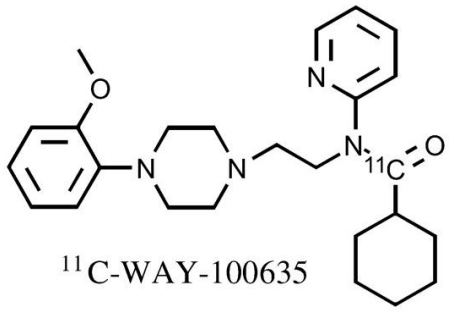

Figure 20 Chemical structure [carbonyl-^{11}C]WAY100635 (taken from Saigal et al. 2006)

4.6.3. General definition of binding potential

Mintun et al. defines the term binding potential BP as the ratio at equilibrium between the specifically bound tracer and the free plus nonspecifically bound tracer and can be expressed as the ratio between receptor density B_{max} (concentration of receptors that are available for binding) and dissociation constant K_D (Mintun et al., 1984, Wu and Carson, 2002) which aims to depict quantification of radiolabeled and specifically bound receptors or proteins in a defined brain tissue region. For a review on further definitions the reader is referred to Gjedde et al. (2005).

Equ. 1 $$BP = \frac{Bmax}{KD}$$

4.6.4. Compartment model, Simplified reference tissue model (SRTM) and Simplified Reference Tissue Model 2 (SRTM2)

The compartment model, originated from the field of pharmacokinetics, is a basic concept for the analysis and the quantitative evaluation of dynamic PET data.

A compartment describes for example an area of the body where the radiotracer is assumed to be homogenous and is a hypothetical apportionment which helps distinguishing between different conditions of the radiotracer and describing relations of these conditions by equations.

The simplest compartment model corresponds to a direct interaction of radiotracer with target tissue *in vitro*, a reaction described by Michaelis and Menten (Michaelis L, 1913), where the ligand L interacts with the receptor R and forms a complex RL (Fig. 21). A detailed description of equations needed for calculation of BP by means of this two compartment model can be found in a publication of Ichise et al. (Ichise et al., 2001)

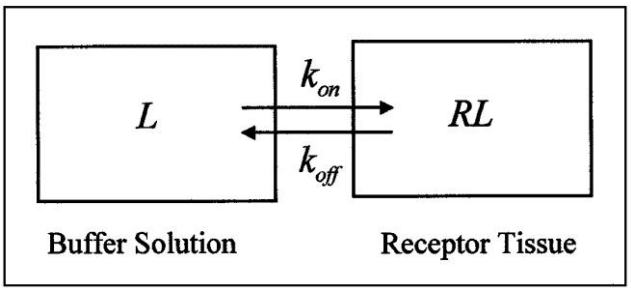

Figure 21 Two compartment model in vitro. L – concentration of unbound radioligand in buffer solution (Bq/mL), RL – concentration of bound radioligand (Bq/mL), kon and koff – association and dissociation kinetic rate constant, respectively (taken from Ichise 2001)

The derivation of the values for B_{max} and K_D in experiments in vivo is more complex due to increasing compartment quantity and to the fact that, unlike in the in vitro system, the in vivo system has an open first compartment (the radioligand is administered intravenously and travels through the heart into the arterial blood system). The radioligand can appear to be freely distributed in the arterial blood plasma, as well as in the brain tissue (i.e. extracellular space), or showing specific (receptor of interest) and non-specific binding (other proteins, fatty acids, etc.). Several tracer kinetic modeling procedures, which provide a mathematical description of radioligand behavior over time, are based on different modifications of the compartment model (Mintun et al., 1984, Frankle et al., 2005, Heiss and Herholz, 2006). The Simplified Reference Tissue Model 2 (SRTM2) was used in this study (Wu and Carson, 2002), which is a further development of the Simplified Reference Tissue Model (SRTM) introduced by Lammertsma et al. in 1996 (Lammertsma and Hume, 1996). The SRTM and SRTM2 models are based on the assumptions of one tissue compartment. The influx and efflux to the tissue ROI with specific and non-displaceable (i.e. free plus nonspecific) binding can be described by the rate constants K_1 and k_2 and the influx and efflux to reference region is denoted as

K'_1 and k'_2. In SRTM three parameters have to be estimated, namely R_1, k'_2 and k_2. R_1 ("relative delivery") is the ratio of K_1 in the region of interest to K_1 in the region of reference (i.e. $R_1 = K_1 / K'_1$). Furthermore binding potential can be obtained through the equation:

Equ. 2

$$BP = \frac{K_1/k_2}{K'_1/k'_2} - 1 = R_1 \frac{k'_2}{k_2} - 1$$

A detailed description of both models was conducted by their authors (Lammertsma and Hume, 1996, Wu and Carson, 2002) and is mentioned in other publications (Frankle et al., 2005, Heiss and Herholz, 2006).

5. METHODS

5.1. Subject characteristics

A total of 43 subjects were analyzed in this study. 18 of them were premenopausal women that have been recruited via advertisements for the study "In vivo imaging of 5-HT_{1A} receptors using PET in patients with anxiety and healthy controls" (EK 318/2002) and 25 postmenopausal women recruited for the study "The influence of hormone replacement therapy on the cerebral serotonin-1A receptor distribution and mood in postmenopausal women" (EC 593/2007) of the Functional, Molecular and Translational Neuroimaging – PET & MRI Group at the Department of Psychiatry and Psychotherapy, Medical University Vienna, Head: A/Prof. PD Dr. Rupert Lanzenberger, MD.

The following inclusion criteria for all subjects were required:

- Signed informed consent form
- Consent not to participate in a study with exposure to radiation within 6 months following the final assessment in this study

Exclusion criteria for all subjects:

- Concomitant major illness
- Concomitant neurological illness
- Current substance abuse
- History of any malign illness
- Implant or stainless steel graft

- Steroid hormone treatment within 3 months prior to the inclusion
- Clinically relevant abnormalities on a general physical examination and routine laboratory screening
- Any exposure to artificial radiation within 3 months prior to the inclusion into the present study

Additional inclusion criteria for premenopausal women:

- age of 18 to 55 years

Additional exclusion criteria for premenopausal women:

- Positive pregnancy test
- Clinically relevant disturbances of the menstrual cycle

The 18 postmenopausal women recruited for the study "The influence of hormone replacement therapy on the cerebral serotonin-1A receptor distribution and mood in postmenopausal women" also had to prove that they were physically healthy by a general physical examination including neurological status, electrocardiogram and a routine laboratory screening.

Additional inclusion criteria for postmenopausal women comprised:

- Age of 45 to 65 years.
- Duration of over 14 months of amenorrhea

Further exclusion criteria for **postmenopausal women**:

- Concomitant psychiatric disorder as assessed by the Structured Clinical Interview for DSM Disorder (SCID), except anxiety disorders or depression
- Treatment with a psychotropic agent targeting 5-HT_{1A} and 5-HT_{2A} receptors or the serotonin transporter
- Concomitant major illness, especially liver disease, disorder of the endocrine system, osteoporosis (when treated with vitamin D)
- Clinically relevant vascular or heart diseases
- Endometriosis
- Cervical smear test PAP > II
- Extensive obesity

- Investigations using PET or SPECT within 10 years prior to the inclusion

All of the subjects had to consent not to participate in PET or SPECT studies with an added equivalence dose of over 15 mSv within 10 years.

5.2. Data acquisition

5.2.1. Image acquisition - Positron Emission Tomography (PET)

Subjects were measured using an ADVANCE full-ring PET scanner (General Electric Medical Systems, Milwaukee, WI, USA) at the Department of Nuclear Medicine, Medical University of Vienna. For quantification of the 5-HT_{1A} receptor binding potential, the radioligand [carbonyl-^{11}C]WAY100635 was chosen. Dynamic PET scans were acquired in 3D mode. The tracer was prepared in a fully automated PET synthesizer (GE Healthcare, Uppsala, Sweden) at the Cyclotron Unit of the PET centre at the Medical University of Vienna. The measurements started simultaneously with the intravenous bolus injection of [carbonyl-^{11}C]WAY100635 in phosphate-buffered saline (injected dose premenopausal subjects 4,7MBq/kg body weight, postmenopausal subjects 3MBq/kg body weight).
For premenopausal women a series of 30 successive time frames (15×1 min, 15×5 min) and for postmenopausal women 50 successive time frames (12x5s, 6x10s, 3x20s, 6x30s, 4x1min, 5x2min, 14x5min) have been performed, each resulting in a total acquisition time of 90 min. Data were reconstructed in a 128×128×35 matrix, with a slice thickness of 4.25 mm using an iterative filtered back-projection algorithm (FORE-ITER). The spatial resolution of the scanner was 4.36 mm full-width at half maximum (FWHM) at the centre of the field of view (FOV).

Only data from the first scan (baseline) without medication from the study “The influence of hormone replacement therapy on the cerebral serotonin-1A receptor distribution and mood in postmenopausal women” were used for the current analysis.

In both groups an automated delineation method of regions of interest as described in Stein et al. has been used (Stein et al., 2008). In short we used a region of interest template based on the anatomical AAL atlas implemented in the SPM8 software (Tzourio-Mazoyer et al., 2002) that has been normalized to a 5-HT_{1A} distribution map in the stereotactic space of the MNI/ICBM brain (Montreal Neurologic Institute/International Consortium for Brain Mapping) and PMOD 3.1 (Meyer et al., 1999). The 5-HT_{1A} distribution map generated by our group represents the mean serotonin-1A

receptor binding potential of 33 healthy subjects (18 females, 15 males) and can be found on the website http://www.meduniwien.ac.at/neuroimaging/mf54_downloads.html.

5.2.2. Quantification of 5-HT$_{1A}$ receptor binding potential

PET data of pre- and postmenopausal women has been normalized to this 5-HT$_{1A}$ distribution map. Decay-corrected time activity curves of 14 a priori defined regions of interest (listed in Table 3, first column) have been used for quantification in PMOD 3.1 [PMOD Technologies Ltd., Zurich, Switzerland (Mikolajczyk et al., 1998)]. The binding potential values of the ROIs were calculated by applying the Simplified Reference Tissue Model 2 (Lammertsma and Hume, 1996, Wu and Carson, 2002) and the kinetic modeling tool of PMOD 3.1. The cerebellar grey matter without the vermis was defined as reference region due to its low binding of [carbonyl-^{11}C]WAY100635 (Hall et al., 1997).

5.2.3. Hormone assays

The analysis of plasma levels of estrogen, progesterone, testosterone, cortisol, DHEA, follicle stimulating hormone (FSH) and luteinizing hormone (LH) from blood samples collected prior to the PET-scans was done by the KIMCL/MUW (Klinisches Institut für Medizinische und Chemische Labordiagnostik).

Blood samples of premenopausal women were collected in follicular phase, i.e. within the first 3–10 days of the menstrual cycle, to ensure exclusion of effects of the menstrual cycle on the 5-HT$_{1A}$ binding potential.

6. STATISTICAL ANALYSIS

The statistical analysis was carried out using the software PASW (SPSS) Statistics 18.0 (SPSS Inc., Chicago). Kolmogorov-Smirnov test was used as a normality test and Levene's test was performed to check for homogeneity of variances. In case that the Bonferroni correction for multiple comparisons had to be applied, an adjusted p-value of 0.0035 was used, otherwise a p-value less than 0.05 was considered as significant. All tests were two-tailed. Mann-Whitney-U tests were performed to investigate if premenopausal women differed from postmenopausal women regarding age, BMI or radiochemical variables.

A mixed model analysis was conducted with the independent variables "menopausal status" and "region of interest", with repeated measures on 14 different ROIs and the dependent variable "5-

HT_{1A} receptor binding potential" to investigate if a significant effect of "menopausal status" on the dependent variable could be detected. Toeplitz covariance structure was used.

A Spearman's Rank Order correlation was run to determine the relationship between the variables "age", "BMI", radiochemical variables ("injected dose", "specific activity"), steroid hormones ("testosterone", "bioavailable testosterone", "progesterone", "estrogen") and the 5-HT_{1A} receptor BP of all subjects taken together. A separate investigation of premenopausal women was carried out by means of Pearson's correlation coefficient between the variables steroid hormones ("testosterone", "bioavailable testosterone", "progesterone", "estrogen") and the 5-HT_{1A} receptor BP. This was also done for postmenopausal subjects, except for the dorsal raphe nucleus, where a non-parametric Spearman's Rank Order correlation had to be applied.

Partial correlations with plasma steroid hormone levels and the 5-HT_{1A} receptor BP were conducted (with different covariates for each investigation).

7. RESULTS

7.1. DESCRIPTIVE STATISTICS

7.1.1. Age, body mass index, injected dose and specific activity of [carbonyl-^{11}C]WAY100635

Premenopausal women were significantly younger and had a significantly lower BMI. The injected dose of [carbonyl-^{11}C]WAY100635 was significantly higher in premenstrual woman, whereas the specific activity was significantly lower (Table 1).

	Premenopausal Mean ± SD	Postmenopausal Mean ± SD	Mann-Whitney U-test U	Exact Sig.
Age (years)	24.11 ± 2.58	55.36 ± 4.76	0	0.000
BMI (kg/m²)	22.65 ± 3.93	25.86 ± 4.31	97	0.001
Injected dose (MBq/kg body weight)	365.54 ± 13.60	210.98 ± 43.32	1	0.000
Specific activity (MBq/kg)	177.32 ± 131.17	391.07 ± 281.42	89	0.001

Table 1 Mean values and standard deviation (Mean ± SD) for age, body mass index (BMI) and radiochemical variables of premenopausal and postmenopausal women.

7.1.2. Plasma levels of steroidal hormones

Blood samples of 14 premenstrual women were collected prior to the PET scan within the follicular phase. For the remaining 4 subjects blood samples collected in follicular phase were not available.

In all 25 postmenopausal subjects blood samples were collected prior to the PET scan. Table 2 shows the mean steroid hormone plasma levels for each group and reference ranges defined by KIMCL. One postmenopausal subject showed a higher estrogen level than defined as reference range for postmenopausal women due to residual ovarian activity.

Premenopausal n=14	**Mean ± SD**	**Range**	**Reference range*[1]**
Progesterone (ng/ml)	0.77 **±** 0.23	0.46 - 1.13	0.5 - 1.0
17ß-Estradiol (pg/ml)	47.78 **±** 24.15	20 - 96	22 - 215
Testosterone (ng/ml)	0.51 ± 0.18	0.19 - 0.94	0.08 - 0.48
Testosterone bioav.*[2] (ng/ml)	0.12 ± 0.01	0.04 - 0.24	0.02 - 0.22
Postmenopausal n=25	**Mean ± SD**	**Range**	**Reference range**
Progesterone (ng/ml)	0.22 ± 0.13	0.10 - 0.60	0.1 - 0.8
17ß-Estradiol (pg/ml)	12.44 ± 6.70	10 - 40	<25
Testosterone (ng/ml)	0.14 ± 0.08	0.03 - 0.36	0.08 - 0.48
Testosterone bioav.*[3] (ng/ml)	0.04 ± 0.00	0.00 - 0.09	0.01 - 0.12

Table 2 Steroid hormone plasma levels of premenopausal women obtained in follicular phase (n=14); *[1] Reference range for follicular phase of the cycle; *[2] Bioavailable testosterone (n=12); reference range defined by KIMCL refers to woman in the age range 20-50a; **Steroid hormone plasma levels of postmenopausal women obtained prior to PET scan (n=25).** *[3] Bioavailable testosterone (n=23); reference range defined by KIMCL refers to woman aged 50 and over. No bioavailable estrogen plasma levels could be obtained.

7.1.3. Distribution and range of the 5-HT$_{1A}$ receptor binding potential in pre- and postmenopausal women

As shown in Table 3, in the second column, the highest 5-HT$_{1A}$ receptor binding potentials occurred in limbic and cortical brain regions, similar to previous studies (Hall et al., 1997, Hornung, 2003, Varnas

et al., 2004, Moller et al., 2009). The mean BP values plus standard deviation and the ranges of the 5-HT_{1A} binding potential of 14 a priori defined regions of interest in pre- and postmenopausal women due to region of interest template (Stein et al., 2008) are illustrated in the third and fourth column. The large between-subject variability 5-HT_{1A} binding potential was similar to those reported in Rabiner et al. and Lundberg et al. (Rabiner et al., 2002, Lundberg et al., 2007).

5-HT_{1A} BP	BP (n=43) Mean ± SD	Premenopausal (n=18) Mean ± SD	Range	Postmenopausal (n=25) Mean ± SD	Range
PHG	5.22 ± 1.41	5.18 ± 1.41	2,05 - 7,72	5.25 ± 1.44	2,75 - 7,42
MTG	4.68 ± 1.34	4.74 ± 1.40	1,56 - 6,31	4.64 ± 1.32	1,98 - 5,28
STG	4.62 ± 1.30	4.62 ± 1.31	1,35 - 5,79	4.61 ± 1.33	1,77 - 4,94
Ins	4.54 ± 1.21	4.65 ± 1.19	1,90 - 7,23	4.45 ± 1.24	2,29 - 6,10
FuG	4.52 ± 1.22	4.60 ± 1.19	1,80 - 6,69	4.45 ± 1.26	2,25 - 6,39
OC	4.51 ± 1.27	4.39 ± 1.15	2,14 - 6,88	4.59 ± 1.37	2,28 - 7,09
ITG	4.38 ± 1.22	4.52 ± 1.24	1,73 - 6,73	4.28 ± 1.21	2,23 - 6,13
GRe	4.25 ± 1.23	4.36 ± 1.25	1,71 - 7,12	4.17 ± 1.23	2,17 - 6,05
CHPC	4.16 ± 1.23	4.19 ± 1.22	1,55 - 5,98	4.13 ± 1.25	1,99 - 5,89
AMY	4.15 ± 1.16	3.90 ± 1.12	1,46 - 6,28	4.33 ± 1.17	2,12 - 5,90
HPC	3.63 ± 1.04	3.82 ± 1.07	1,31 - 5,44	3.49 ± 1.10	1,84 - 5,01
ACC	3.50 ± 1.01	3.62 ± 1.04	1,30 - 5,74	3.43 ± 1.00	1,66 - 4,95
DRN	2.34 ± 1.02	2.15 ± 0.62	1,08 - 3,67	2.48 ± 1.23	1,08 - 5,84
MRN	2.19 ± 0.78	2.19 ± 0.78	0,73 - 3,88	2.19 ± 0.80	1,01 - 3,98

Table 3 First column 14 a priori defined regions of interest listed in descending order according to 5-HT_{1A} BP value. **Second column** Mean regional 5-HT_{1A} BP of pre- and postmenopausal women taken together (SD – standard deviation). **Third and fourth column** mean 5-HT_{1A} BP value **±** SD and range is given for each subject group; parahippocampal gyrus (**PHG**), middle temporal gyrus (**MTG**), superior temporal gyrus (**STG**), insula (**Ins**), fusiform gyrus (**FuG**), olfactory cortex (**OC**), inferior temporal gyrus (**ITG**), gyrus rectus (**GRe**), caput hippocampi (**CHPC**), amygdala (**AMY**), hippocampus (**HPC**),anterior cingulate cortex (**ACC**), dorsal raphe nucleus (**DRN**), median raphe nucleus (**MRN**).

7.2. ANALYTICAL STATISTICS

7.2.1. Effect of menopausal status on the 5-HT_{1A} RBP of pre- and postmenopausal women

Contrary to expectations, the conducted mixed model ANOVA did not reveal a significant effect of menopausal status on 5-HT_{1A} receptor BP, F(1, 41,756)=.078, p=.0781. The main effect of region of interest (ROI) was significant F(13, 117,764)=107.746, p=.000. The interaction term menopausal status by region of interest was significant, F(6, 143.764)=2.449, p=.028, therefore posthoc t-tests in each region of interest were performed.

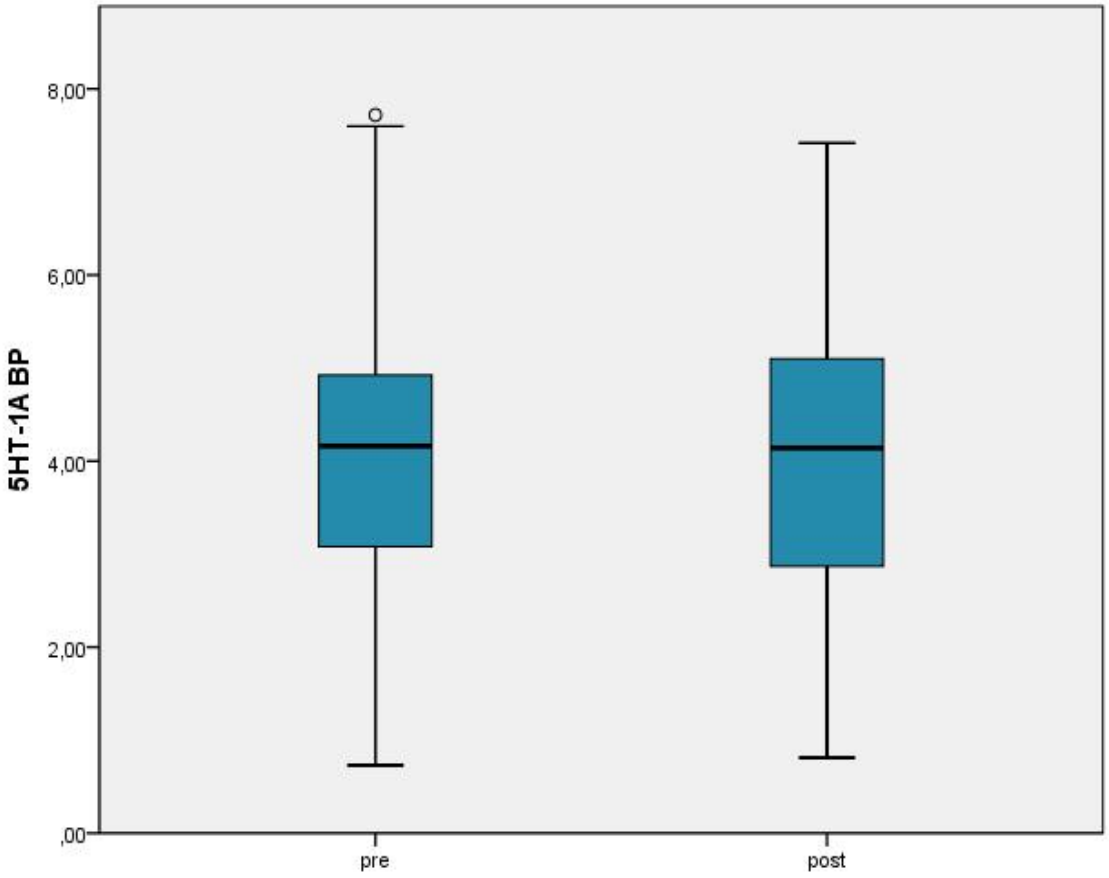

Figure 22 Comparison of the median 5-HT$_{1A}$ RBPs of pre- and postmenopausal woman - boxplot The boxplot shows an approximately equal median of 5-HT$_{1A}$ BP in the two defined groups.

7.2.2. Regional differences in 5-HT$_{1A}$ RBP between pre- and postmenopausal women

Regional differences in BP between pre- and postmenopausal women by means of independent samples t-tests were performed in each region of interest. No significant differences in any investigated region were found (see Table 7 in the annex).

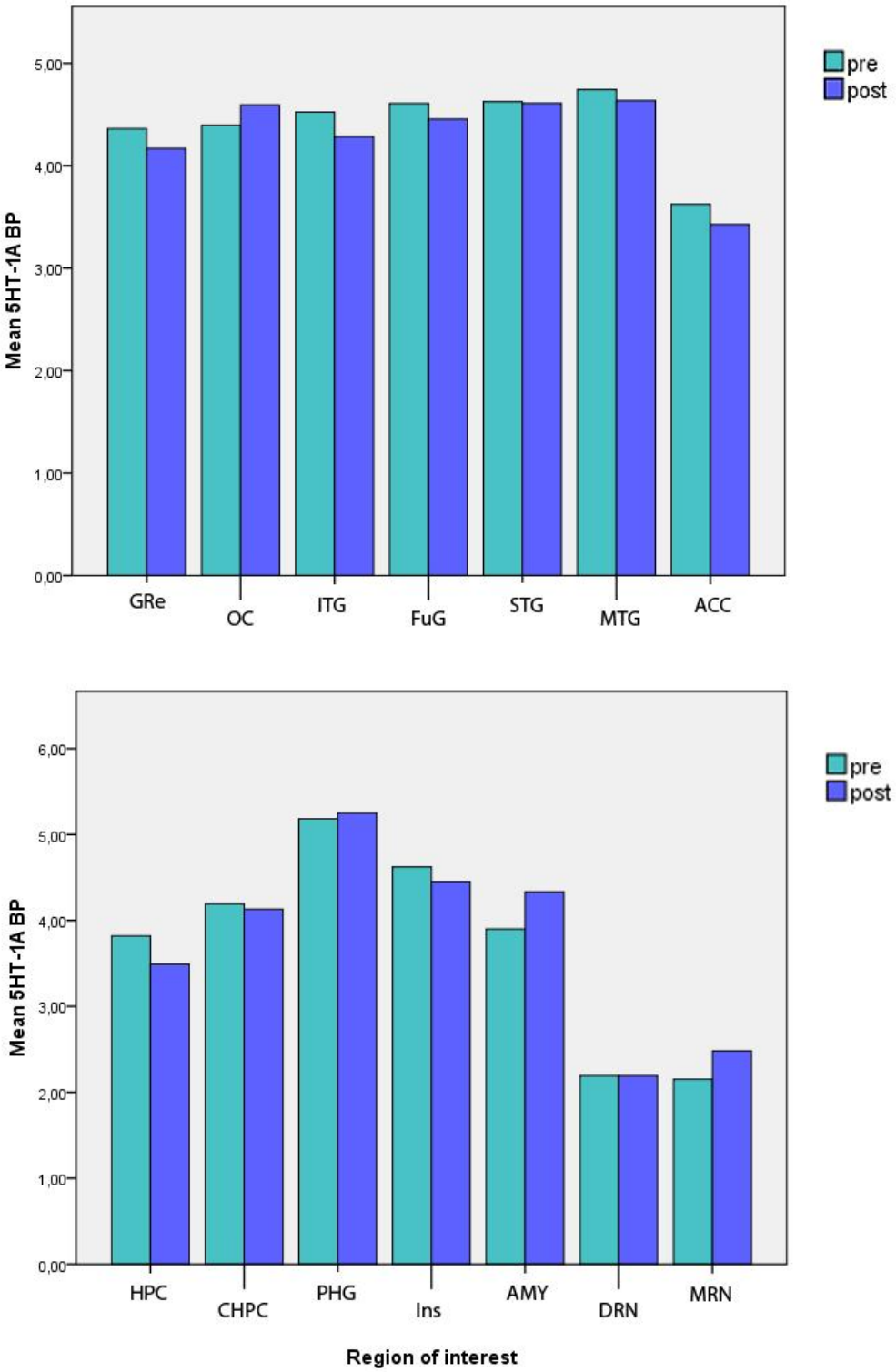

Figure 23 Comparison of mean 5-HT$_{1A}$ RBP of pre- and postmenopausal women in each region of interest; Gre – gyrus rectus, **OC** – olfactory cortex, **ITG** – inferior temporal gyrus, **FuG** – fusiform gyrus, **STG** – superior temporal gyrus, **MTG** – middle temporal gyrus, **ACC** – anterior cingulate cortex, **HPC** – hippocampus, **CHPC** – caput hippocampi, **PHG** – parahippocampal gyrus, **Ins** – insula, **AMY** – amygdala, **DRN** – dorsal raphe nucleus, **MRN** – median raphe nucleus

7.2.3. Correlation of 5-HT_{1A} RBP with age, body mass index and radiochemical variables in pre- and postmenopausal women

In contrast to earlier findings no evidence of a relationship between age and 5-HT_{1A} BP was detected by means of Spearman's rank correlation coefficient of both groups taken together. A recent investigation by Erritzoe et al (2010) demonstrated an inverse relationship between 5HTT and body mass index. In the current study no relationship between BMI and BP nor between the radiochemical variable "injected dose" was traced. Table 8 in the annex contains results for each investigated region of interest.

7.2.4. Correlation of 5-HT_{1A} RBP and steroid hormone plasma levels in pre- and postmenopausal women

All 14 ROIs were investigated separately by means of Spearman's rank correlation coefficient in pre- and postmenopausal women taken together. In the amygdala a medium effect of progesterone [r(41)= -0.346, p=0.031], testosterone [r(41)= -0.405, p=0,011] and bioavailable testosterone [r(41)= -0.443 p=0.008] on BP was detected. A negative correlation with bioavailable testosterone was also shown in the olfactory cortex and the raphe nuclei, but those effects disappeared after Bonferroni correction for multiple comparisons (adjusted p<0,0035). For detailed information see Table 9 in the annex.

7.2.5. Correlation of 5-HT_{1A} RBP and steroid hormone plasma levels in premenopausal woman

Pearson's product moment correlation did not reveal any relationship in the investigated regions of interest. Detailed results can be found in Table 10 in the annex.

Partial correlations of steroid hormone plasma levels and 5-HT_{1A} BP did not reach the level of significance, therefore the detailed results are not shown in the result section.

7.2.6. Correlation of 5-HT_{1A} BP and steroid hormone plasma levels in postmenopausal woman

Interestingly a negative correlation between testosterone, as well as bioavailable testosterone plasma levels, and binding potential in all investigated regions of interest occurred in postmenopausal subjects. In case of testosterone, only the binding potential in the dorsal raphe

nucleus survived the Bonferroni correction of multiple comparisons. As Table 4 shows, in case of bioavailable testosterone, ten out of fourteen investigated ROIs remained significant (adjusted p<0.0035).

ROI	Estrogen		Progesterone		Testosterone		BAT	
	r	p	r	p	r	p	r	p
GRe	0.129	0.538	-0.060	0.775	-0.450*	0.024	-0.532**	0.009
OC	0.050	0.811	-0.162	0.439	-0.502*	0.010	-0.604**	0.002*[2]
ITG	0.048	0.819	-0.108	0.608	-0.472*	0.017	-0.573**	0.004
FuG	0.137	0.515	-0,163	0.435	-0.506*	0.010	-0.604**	0.002*[2]
STG	0.120	0.567	-0.146	0.486	-0.525**	0.007	-0.613**	0.002*[2]
MTG	0.103	0.624	-0.135	0.521	-0.482*	0.015	-0.574**	0.004
ACC	0.064	0.762	-0.112	0.594	-0.539**	0.005	-0.635**	0.001*[2]
HPC	0.121	0,564	-0.216	0.300	-0.500*	0.011	-0.596**	0.003*[2]
CHPC	0.164	0.433	-0.197	0.345	-0.485*	0.014	-0.579**	0.004
PHG	0.166	0.427	-0.235	0.259	-0.509**	0.009	-0.584**	0.003*[2]
Ins	0.112	0.593	-0,137	0.515	-0.509**	0.005	-0.604**	0.002*[2]
AMY	0.117	0.577	-0.240	0.249	-0.551**	0,004	-0.590**	0.003*[2]
DRN*[1]	0.120	0.567	-0.364	0.074	-0.577**	0.003*[2]	-0.618**	0.002*[2]
MRN	-0.104	0.622	-0.162	0.438	-0.532**	0.006	-0.652**	0.001*[2]

Table 4 Correlation of 5-HT$_{1A}$ BP in postmenopausal women and steroidal hormones by means of Pearson product moment correlation. **BAT** – bioavailable testosterone; gyrus rectus (**GRe**), olfactory cortex (**OC**), inferior temporal gyrus (**ITG**), fusiform gyrus (**FuG**), superior temporal gyrus (**STG**), middle temporal gyrus (**MTG**), anterior cingulate cortex (**ACC**), hippocampus (**HPC**), caput hippocampi (**CHPC**), parahippocampal gyrus (**PHG**), insula (**Ins**), amygdala (**AMY**), dorsal raphe nucleus (**DRN**), median raphe nucleus (**MRN**). ***[1]** Spearmen's rank correlation coefficient, *[2] survived the Bonferroni correction for multiple comparisons with an adjusted p<0.0035, * correlation is significant at the 0.05 level (2-tailed), ** correlation is significant at the 0.01 level (2-tailed)

In order to assess the impact on 5-HT$_{1A}$ binding potential that can be solely attributed to testosterone and bioavailable testosterone, respectively, the variables estrogen and progesterone were partialled out. Table 5 presents results obtained from partial correlation of testosterone, as well as bioavailable testosterone, with the covariates estrogen and progesterone included into the

model. Only in bioavailable testosterone analysis seven ROIs survived the Bonferroni correction, namely the orbitofrontal cortex, inferior temporal gyrus, fusiform gyrus, superior temporal gyrus, anterior cingulate cortex, insula and the median raphe nucleus. Figure 24 shows a partial regression plot in anterior cingulate cortex, further partial regression plots can be found in the annex (section 13.5). Partial correlations of other steroidal hormones were not significant therefore the detailed results are not shown in the result section.

ROI	Testosterone		BAT	
	r	p	r	p
GRe	-0.504*	0.014	-0.594**	0.004
OC	-0.503*	0.014	-0.612**	0.003*[1]
ITG	-0.499*	0.015	-0.612**	0.003*[1]
FuG	-0.507*	0.014	-0.622**	0.003*[1]
STG	-0.540**	0.008	-0.644**	0.002*[1]
MTG	-0.494*	0.017	-0.597**	0.004
ACC	-0.579**	0.004	-0.680**	0.001*[1]
HPC	-0.469*	0.024	-0.569**	0.007
CHPC	-0.461*	0.027	-0.550*	0.010
PHG	-0.469*	0.024	-0.570**	0.007
Ins	-0.564**	0.005	-0.633**	0.002*[1]
AMY	-0.519*	0.011	-0.552**	0.009
DRN	-0.476*	0.022	-0.559**	0.008
MRN	-0.540**	0.008	-0.674**	0.001*[1]

Table 5 Partial correlation of 5-HT$_{1A}$ BP and testosterone, as well as bioavailable testosterone, in postmenopausal women after controlling for estrogen and progesterone, *[1] survived the Bonferroni correction for multiple comparisons with an adjusted p<0.0035; * correlation is significant at the 0.05 level (2-tailed), ** correlation is significant at the 0.01 level (2-tailed)

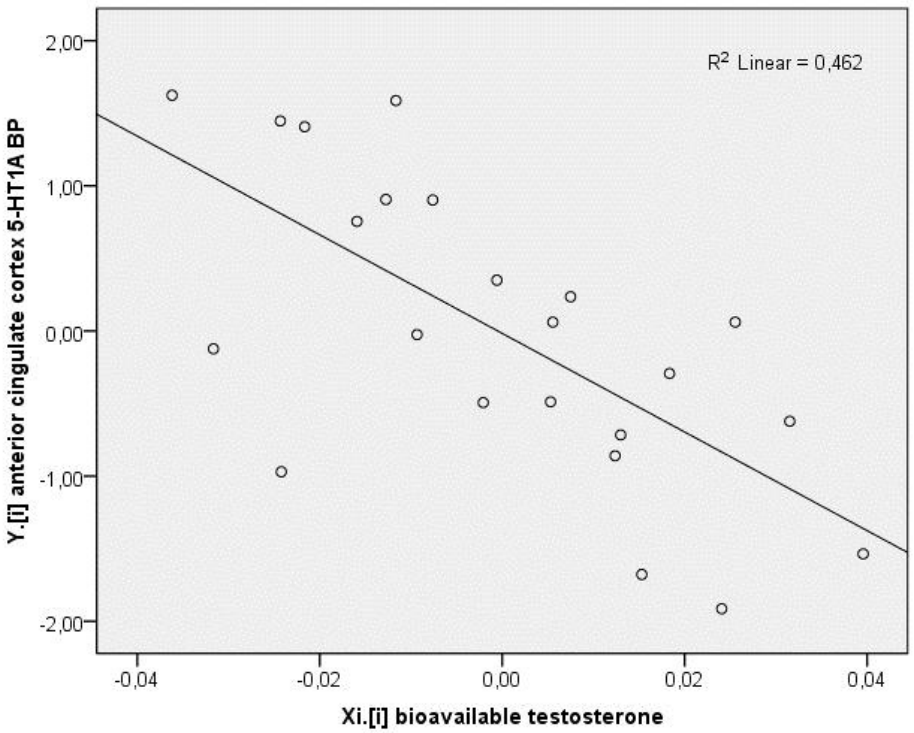

Figure 24 Partial regression plot of the 5-HT1A BP in anterior cingulate cortex of postmenopausal subjects and bioavailable testosterone adjusted for estrogen and progesterone
Note:

$Y_{.[i]}$ = residuals from regressing Y (the response variable **5-HT1A BP**) against independent variables **estrogen and progesterone** except Xi **bioavailable testosterone**;

$X_{i.[i]}$ = residuals from regressing X_i **bioavailable testosterone** against the independent variables **estrogen and progesterone**

8. DISCUSSION

Contrary to expectations, this study did not reveal differences of 5-HT_{1A} BP due to menopausal status in pre- and postmenopausal women. To date no other study focussing on the comparison of 5-HT_{1A} BP in pre- and postmenopausal women *in vivo* has been published. In addition, no human post mortem surveys regarding this issue are available.

Despite the common prescription of steroidal hormones, either in the context of contraception or hormone replacement therapy, little is known about their effect on serotonergic neurotransmission in humans, in particular only a few studies are available regarding the main inhibitory 5-HT_{1A} receptor in women. More recently, literature emerged that offers contradictory findings compared to existing studies in rodents or non-human primates. Our group observed a positive correlation of 5-HT_{1A} BP

and 17ß-estradiol plasma levels in the raphe nuclei (Witte et al., 2009) whereas Moses-Kolko et al. demonstrated a positive relationship in postsynaptic brain areas in females (Moses-Kolko et al., 2011). Progesterone was shown to act suppressive on the 5-HT_{1A} BP, but this strong relationship was solely true for human males (Lanzenberger et al., 2011). Only one observer identified an interrelation of testosterone plasma levels in several cortical brain areas of premenopausal women (Witte et al., 2009).

Three human studies should be mentioned at this point which are not directly related to 5-HT_{1A} BP in pre- or postmenopausal women but giving an insight into steroidal hormone influences on serotonergic transmission in general.

Jovanovic et al. examined patients suffering from premenstrual dysphoric disorder and observed a non-significantly lower 5-HT_{1A} receptor BP in the DRN in the follicular phase in healthy controls (Jovanovic et al., 2006) compared to the luteal phase of the menstrual cycle. A second investigation by the same group three years later again traced a non-significant trend in the same brain region. The author suggests, as an explanation for it, the absence of influences of menstrual cycle phases on 5-HT_{1A} receptors.

The third one, a recent survey of a similar study design to the current investigation, was conducted focussing on 5-HT_{2A} receptors. At the 25th International Symposium on Cerebral Blood Flow, Metabolism, and Function and 10th International Conference on Quantification of Brain Function with PET in May 2011 Kroll et al. presented a poster of a comparison of cortical [^{18}F]altanserin binding potential between pre- and postmenopausal women. In a sample of 16 premenopausal and 8 postmenopausal women no significant differences in 5-HT_{2A} receptor BP in cortical brain regions were detected. It would have been more interesting to include an investigation of the influence of steroidal hormone plasma levels on 5-HT_{2A} receptor BP in this subject sample. However, only prolactin plasma levels were available and they were not correlated to cortical 5-HT_{2A} RBP in pre- and postmenopausal women.

In addition, the 5-HT_{2A} BP was investigated in premenopausal women (follicular phase n=9, luteal phase n=7), similar to Jovanovic et al., throughout the menstrual cycle. Progesterone plasma levels were significantly higher in the luteal phase, and a slight increase of [^{18}F]altanserin binding potential in luteal phase of the menstrual cycle was shown but did not reach the level of significance (Kroll, 2011).

However, these three studies have to be interpreted with caution due to the small sample sizes and since no further study regarding this issue in an adequate sample was published, the influences of menstrual cycle phases on 5-HT_{1A} receptor BP, as well as the impact of menopausal status on 5-HT_{2A} BP, remain unclear.

Hence, possibilities of further comparison to publications in the past are restricted to studies in rodents or non-human primates. The research to date has tended to focus on rats rather than on humans, non-human primates or mice.

A consolidated view on these research findings indicates that **presynaptic 5-HT$_{1A}$ receptors and mRNA levels** were mostly **decreased** by **estrogen** administration in **rats and non-human primates**. This stands in contrast to studies in **humans** which show either a **positive relationship** between these two variables in raphe nuclei of premenopausal women (Witte et al., 2009) or **no correlation** (Moses-Kolko et al., 2011). The present study did not trace this positive interrelation, neither in premenopausal women, nor in the study sample of postmenopausal woman. The sample size of premenopausal woman was exactly the same compared to Witte et al., the age range was very similar and in both surveys the subjects were measured in follicular phase of the menstrual cycle and had to meet the same inclusion criteria. One possible explanation for these diverging results would be a mismatch in proportion of subjects measured in early follicular to late follicular phase where estradiol increases to periovulatory levels.

Previous animal studies regarding **postsynaptic** 5-HT$_{1A}$ receptors have provided **no definitive answer** to the question of whether estrogens act suppressive or stimulating since the receptor density was either **decreased** (Osterlund et al., 2000, Le Saux and Di Paolo, 2005), **no alteration** could be detected (Flugge et al., 1999, Jackson and Etgen, 2001, Landry and Di Paolo, 2003, Le Saux and Di Paolo, 2005) or even **increased** receptor binding has been demonstrated (Flugge et al., 1999, Moses-Kolko et al., 2011). A recent human study revealed a **positive correlation** with greatest associations in lateral orbitofrontal, occipital and pregenual cortices (Moses-Kolko et al., 2011). The absence of a relationship of estradiol and 5-HT$_{1A}$ BP in the current study is in line with Witte et al. 2009 (Witte et al., 2009) and several animal examinations (Flugge et al., 1999, Jackson and Etgen, 2001, Landry and Di Paolo, 2003, Le Saux and Di Paolo, 2005).

This study could not confirm findings of a decreased BP due to increased **progesterone** plasma levels in females as seen in males (Lanzenberger et al., 2011), in non-human primates (Henderson and Bethea, 2008) or rodents (Lu et al., 1999), hence the absence of an effect attributed to progesterone in females supports previous research in non-human primates (Gundlah et al., 1999) or humans (Moses-Kolko et al., 2011).

In contrast to the findings of Witte et al. where a positive correlation between BP and **testosterone** plasma levels appeared in frontal cortices and the anterior cingulate cortex of premenopausal women (Witte et al., 2009) the current study traced an inverse relationship in cortical as well as

limbic brain regions and the median raphe nucleus in women after menopause. Due to the fact that the current study revealed this effect in postmenopausal woman, when plasma levels of estrogen and progesterone cease and therefore the interrelation of other steroidal hormones is extenuated, these findings are more likely to accurately reflect the influence of testosterone on 5-HT_{1A} receptors. Corresponding studies in rats indicated that testosterone is capable to either increase 5-HT_{1A} BP (Flugge et al., 1999) or to decrease 5-HT_{1A} mRNA (Zhang et al., 1999).

If it is assumed that premenopausal women show increased estrogen, progesterone and testosterone plasma levels (E↑, P↑, T↑) compared to postmenopausal women, several different explanations can be considered for the results of the current study.

When assuming that the recent study findings regarding the effect of estrogen on 5-HT_{1A} receptors are correct and in addition increased estrogen plasma levels lead to an increased BP (Moses-Kolko et al., 2011), no significant effect of progesterone in females has to be considered (Moses-Kolko et al.,2011, Witte et al., 2010, current study), it is assumed that testosterone decreases BP (current study) and no age effect is presumed, the net impact on 5-HT_{1A} receptors remains balanced (see Table 6, 1 - premenopausal).

A similar situation would occur in the case that progesterone is not only negatively correlated with 5-HT_{1A} BP in animals (Lu et al., 1999, Bethea et al., 2002a, Henderson and Bethea, 2008) or in males (Lanzenberger et al., 2011), but also in females and if additionally an inverse relationship of age and BP (Tauscher et al., 2001, Costes et al., 2005, Moller et al., 2007, Moller et al., 2009) is assumed (see Table 6, 2 - premenopausal).

If estrogen is negatively correlated to 5-HT_{1A} BP, or when assuming that the testosterone effect can be seen equal to an estrogen effect due to conversion of testosterone into estrogen in the brain tissue (E=T → ↓BP) a third explanatory model is most probable where an additional unknown positive correlated covariate finally balances out all the effects taken together (see Table 6, 3 - premenopausal).

1)	premenopausal	E↑ → **↑BP**	postmenopausal	E↓ → **↓BP**
		P↑ → **↔BP**		P↓ → **↔BP**
		T↑ → **↓BP**		T↓ → **↑BP**
		age↓ → **↔BP**		age↑ → **↔BP**

2)	premenopausal	E↑ → **↑BP** P↑ → **↓BP** T↑ → **↓BP** age↓ → **↑BP**	postmenopausal	E↓ → **↓BP** P↓ → **↑BP** T↓ → **↑BP** age↑ → **↓BP**
3)	premenopausal	E↑ → **↓ BP** P↑ → **↓ or ↔ BP** T↑ → **↓ BP** age↓ → **↑ or ↔ BP** ? → **↑**	postmenopausal	E↓ → **↑ BP** P↓ → **↑ or ↔ BP** T↓ → **↑ BP** age↑ → **↓ or ↔ BP** ? → **↓**

Table 6 Explanatory models for balanced BP in the comparison of pre- and postmenopausal women

One question that needs to be asked, however, is whether the extent of detected differences in steroid hormone plasma levels is capable of indicating alterations. PET scans and blood samples of premenopausal women were conducted or collected in follicular phase of the menstrual cycle, when estrogen and progesterone are usually lower than in luteal phase or during periovulatory period. To date only two human studies were conducted which examine effects of menstrual cycle phases on serotonin receptors and both could not trace significant differences (Jovanovic et al., 2009, Kroll, 2011).

8.1. Limitations

8.1.1. Inter-subject variability

In this sample of subjects a large inter-subject variability in 5-HT$_{1A}$ BP occurred (see Table 3), similar to other human PET studies (Rabiner et al., 2002, Lundberg et al., 2007, Moller et al., 2007). This phenomenon complicates an appropriate interpretation of results from studies which are not based on a longitudinal design. Under these circumstances hormonal influences on serotonergic 5-HT$_{1A}$ receptor BP could be disguised because all premenopausal women with a large inter-subject variability in 5-HT$_{1A}$ BP as one group are opposed to a group of postmenopausal women in a cross-sectional study design. Due to high costs of PET measurements and ethical considerations this study

design is still commonly used (Parsey et al., 2002, Stein et al., 2008, Martinez et al., 2009, Moses-Kolko et al., 2011).

8.1.2. Age effect on 5-HT$_{1A}$ receptors

A further limitation is the difference in age of these two defined groups. Several studies suggest an age effect on 5-HT$_{1A}$ receptors. Tauscher et al. demonstrated a global decrease of BP by 10% per decade (Tauscher et al., 2001), Möller et al. found a decrease of only 3-4% per decade (Moller et al., 2007) and in 2009 his group again showed a regional decrease of 5-HT$_{1A}$ receptors (Moller et al., 2009). Similar results have been reported by Costes et al. (53 subjects, age range 20-70 years, 27 females) with a decrease of 3.6% per decade (Costes et al., 2005), but this was only true for females, whereas Meltzer et al. found a decrease of 5-HT$_{1A}$ receptor BP only in male subjects (Cidis Meltzer et al., 2001). Another study with a large sample size was conducted by Rabiner et al. 2002 (61 subjects, age range 24-53 years) who investigated only male subjects revealing no difference in 5-HT$_{1A}$ BP with increasing age (Rabiner et al., 2002). Recently Moses-Kolko et al. published data with the largest group of subjects available to date (71 subjects, age range 20-80 years, 34 females) showing a small inverse relationship between postsynaptic BP and age in men and an unanticipated small to medium increase in postsynaptic BP with age in women (Moses-Kolko et al., 2011).
In the current study no correlation of age and BP in a sample of 43 females was detected which is consistent with the findings of Meltzer et al. 2001 (11 female subjects) and Parsey et al. (12 female subjects) who did not identify significant relationships between these two variables (Cidis Meltzer et al., 2001, Parsey et al., 2002). Therefore it was not possible to partial this potentially confounding variable out. However, it cannot be ruled out that age did have an impact on 5-HT$_{1A}$ receptor BP and obscured the influence of the other covariates.

These considerations, however, remain speculative since discrimination between the separate effects of each hormone is not feasible in a cross-sectional study design. The interplay of steroidal hormones becomes even more complex when considering that in the brain tissue testosterone is converted by P450-aromatase to 17ß-estradiol (Lephart, 1996, Mukai et al., 2006) or by 5α-reductase to dihydrotestosterone DHT (Lephart et al., 2001). Also dehydroepiandrosterone sulfate DHEAS, a precursor of estrogen and testosterone, was identified to be connected to hypothalamic–pituitary–adrenal (HPA) axis and serotonergic neurotransmission, in particular a positive correlation to 5-HT$_{1A}$ receptors in hypothalamus was shown (Moser et al., 2010). Beside the suppressive effect of cortisol (Flugge et al., 1998) other covariates might have an influence that were not identified to date and also genetic polymorphisms and epigenetics, as suggested by Parsey et al. (Parsey, 2010), as well as

many other authors, have to be taken into account in future studies. The net effect of steroids on serotonergic neurotransmission is also dependent on many other factors not mentioned in detail in the current study (e.g. the presence of enzymes involved in synthesis and degradation of hormones and corresponding receptors, and hence the resulting available endogenous ligand and receptor distribution). These are already discussed elsewhere (Ramirez and Zheng, 1996, Simon et al., 1998, Bethea et al., 2002a, Le Saux and Di Paolo, 2005, Lundberg et al., 2007, Feng et al., 2010, Grassi et al., 2010, Parsey, 2010).

9. CONCLUSION

Returning to the hypotheses posed at the beginning of this study, it is now possible to state that in our sample of 43 subjects menopausal status had no effect on 5-HT_{1A} BP. A post-hoc analysis in each investigated region of interest revealed no significant differences in 5-HT_{1A} BP between pre- and postmenopausal women. However, these findings are limited by the use of a cross-sectional study design and the large inter-subject variability in 5-HT_{1A} receptor binding potential. Identification of subgroups (e.g. genetic polymorphisms leading to diverging BP; a possible cause of the inter-subject variability) and a separate comparison of these subjects would help us to establish a greater degree of accuracy on this matter.

The same limitations are source of weakness in the current study when investigating influences of steroidal hormone plasma levels on 5-HT_{1A} RBP. A negative correlation of 5-HT_{1A} RBP and testosterone was traced in the dorsal raphe nucleus of postmenopausal women. In ten regions of interest (olfactory cortex, fusiform gyrus, superior temporal gyrus, anterior cingulate cortex, hippocampus, parahippocampal gyrus, insula, amygdala, dorsal raphe nucleus, median raphe nucleus) an inverse relationship of bioavailable testosterone and 5-HT_{1A} BP in the same subgroup was shown. Given the fact that plasma levels of estrogen and progesterone disguise the impact of testosterone on 5-HT_{1A} RBP and correlations of testosterone were shown in postmenopausal subjects, in which estrogen and progesterone plasma levels declined, the present study provides additional evidence with respect to testosterone effects that might be more accurate compared to previous studies. However, since this is the first evidence of a suppressive effect of testosterone in humans this issue needs to be reexamined.

When assuming that testosterone is converted into estrogen in the brain tissue and they both have a suppressive effect, an increase of these hormones leads to decreased presynaptic and postsynaptic

receptors resulting in decreased autoinhibition and increased serotonergic firing rate, as well as a region specific modulation of glutaminergic neurons, with an excitatory net effect on the serotonergic system.

As mentioned above there is a definite need for future trials that take into account the causation of large inter-subject variabilities. A longitudinal study design, as used in the ongoing study of our group, where the influence of hormone replacement therapy on 5-HT_{1A} RBP in postmenopausal subjects is being investigated, will add substantially to our understanding of the influence of steroidal hormones on serotonergic neurotransmission, and hence further elucidate the suggested link between steroidal hormones and psychiatric diseases, like depression.

10. LIST OF FIGURES

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12. LIST OF 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
AR	androgen receptor
BMI	body mass index
BP	binding potential
BP_{ND}	non-displaceable binding potential
BT	bioavailable testosterone
CSF	cerebrospinal fluid
DHT	dihydrotestosterone
E	estrogen
ER	estrogen receptor
GABA	γ-aminobutyric acid
GBq	gigabecquerel
HPA	hypothalamic–pituitary–adrenal axis
HPLC	high performance liquid chromatography
LH	luteinizing hormone
LNAAT	large neutral amino acid transporter
MAO	monoamine oxidase
MBq	megabecquerel
MRI	magnetic resonance imaging
PCPA	parachlorphenylalanin
PET	positron emission tomography
PR	progesterone receptor
ROI	region of interest
SBP	serotonin binding protein
SRTM	simplified reference tissue model
T	testosterone

TAC	time activity curve
TPH	tryptophan hydroxylase
TRP	tryptophan
VMAT	vesicular monoamine transporter

13. ANNEX

13.1. Serotonin system, sagittal section

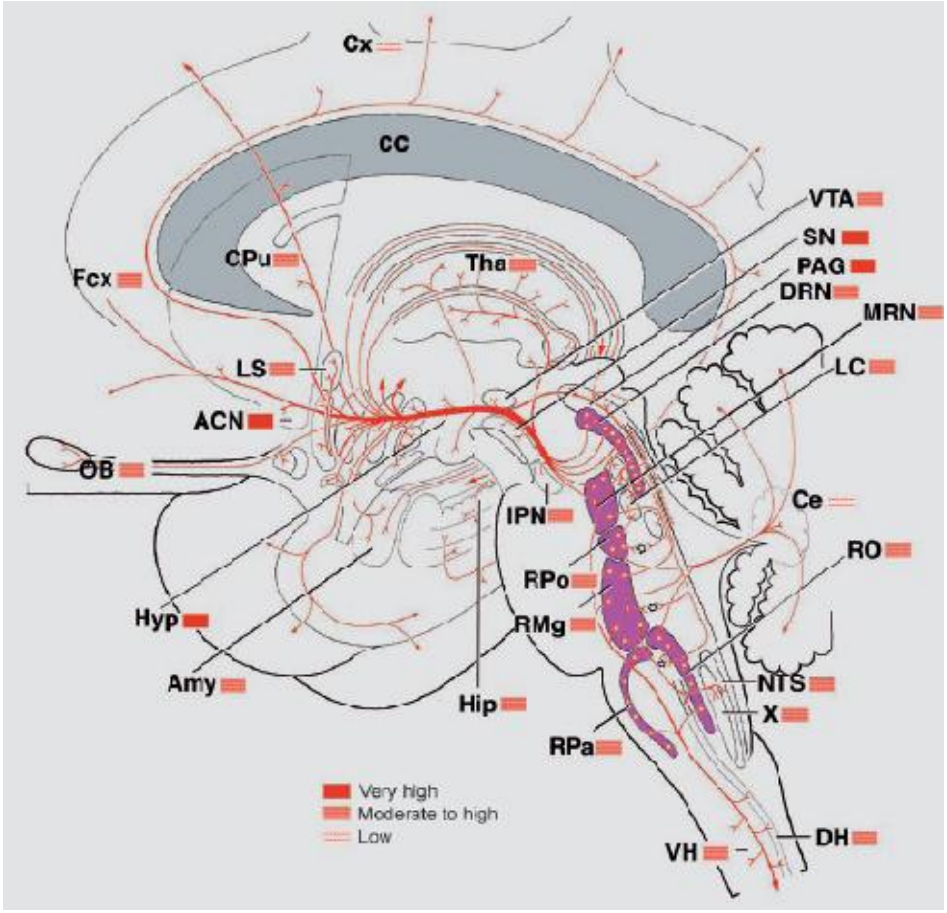


Figure 5 Serotonin system, sagittal section showing raphe nuclei (purple, located in brain stem) and projections (axons illustrated red) to cortical, limbic brain areas, cerebellum and spinal cord. Colored boxes denote densities of serotonergic axonal networks.

X - dorsal motor nucleus of the vagus nerve; **ACN** - nucleus accumbens; **Amy** - amygdala; **cc** - corpus callosum; **Ce** - cerebellum; **Cpu** - caudate-putamen; **Cx** - cortex; **DH** - dorsal horn spinal cord; **DRN** - dorsal raphe nucleus; **Fcx** - frontal cortex; **Hip** - hippocampus; **Hyp** - hypothalamus; **IPN** - interpeduncular nucleus; **LC** - locus coeruleus; **LS** - lateral septum; **MRN** - median raphe nucleus; **NTS** - nucleus of the solitary tract; **OB** - olfactory bulb; **PAG** - periaqueductal gray; **RMg –** nucleus raphe magnus; **RO** - nucleus raphe obscurus; **Rpa** - raphe pallidus; **Rpo** - nucleus raphe pontis; **SN** - subtantia nigra; **Tha** - thalamus; **VH** - ventral horn; **VTA** - ventral tegmental area (Taken from Charnay et al. 2010)

13.2. Regional differences in 5-HT_{1A} receptor BP between pre- and postmenopausal women

Region of interest	Premenopausal Mean ± SD	Postmenopausal Mean ± SD	t-test t	Sig.
Gyrus rectus	4.36 ± 1.25	4.17 ± 1.23	0.51	0.62
Olfactory cortex	4.39 ± 1.15	4.59 ± 1.37	-0.50	0.62
Inferior temporal gyrus	4.52 ± 1.24	4.28 ± 1.21	0.64	0.53
Fusiform gyrus	4.60 ± 1.19	4.45 ± 1.26	0.41	0.69
Temporal pole: sup. temporal gyrus	4.62 ± 1.31	4.61 ± 1.33	0.04	0.97
Temporal pole: middle temporal gyrus	4.74 ± 1.40	4.64 ± 1.32	0.26	0.80
Anterior cingulate cortex	3.62 ± 1.04	3.43 ± 1.00	0.62	0.54
Hippocampus	3.82 ± 1.07	3.49 ± 1.102	1.03	0.31
Caput hippocampi	4.19 ± 1.22	4.13 ± 1.25	0.17	0.87
Parahippocampal gyrus	5.18 ± 1.41	5.25 ± 1.44	-0.15	0.88
Insula	4.65 ± 1.19	4.45 ± 1.24	0.54	0.59
Amygdala	3.90 ± 1.12	4.33 ± 1.17	-1.22	0.23
Median raphe nucleus	2.19 ± 0.78	2.19 ± 0.80	-1.05	0.30
Dorsal raphe nucleus	2.15 ± 0.62	2.48 ± 1.23	219	0.88*

Table 7 Regional differences in 5-HT_{1A} BP between pre- and postmenopausal women. The independent-samples t-tests revealed no significant regional differences between pre- and postmenopausal woman. *Mann-Whitney-U test was chosen for DRN due to its deviation from normal distribution in postmenopausal women.

13.3. Correlation of 5-HT_{1A} BP with age, body mass index and radiochemical variables in pre- and postmenopausal women

Region of interest	Age		BMI		Injected dose	
	r	p	r	p	r	p
Gyrus rectus	-0,003	0,986	-0,004	0,980	0,172	0,301
Olfactory cortex	0,111	0,508	0,071	0,672	0,086	0,608
Inferior temporal gyrus	-0,057	0,733	-0,037	0,827	0,201	0,227
Fusiform gyrus	-0,039	0,816	-0,015	0,930	0,164	0,324
Superior temporal gyrus	0,000	1,000	0,016	0,926	0,150	0,368
Middle temporal gyrus	0,049	0,771	0,033	0,842	0,164	0,326
Anterior cingulate cortex	-0,073	0,665	-0,024	0,887	0,212	0,201
Hippocampus	-0,170	0,308	-0,073	0,665	0,246	0,136
Caput hippocampi	-0,071	0,674	0,023	0,892	0,119	0,477
Parahippocampal gyrus	0,090	0,593	0,068	0,683	0,079	0,636
Insula	-0,074	0,661	-0,070	0,677	0,144	0,387
Amygdala	0,275	0,095	0,207	0,213	-0,093	0,580
Dorsal raphe nucleus	0,072	0,666	-0,114	0,496	0,080	0,631
Median raphe nucleus	0,052	0,757	-0,055	0,745	0,030	0,859

Table 8 Correlation of BP and age, BMI or injected dose of [carbonyl-^{11}C]WAY100635 and 5-HT_{1A} receptor BP by means of Spearmen's rank correlation coefficient in both groups taken together.

13.4. Correlation of 5-HT$_{1A}$ BP and steroid hormone plasma levels in pre- and postmenopausal women

ROI	Estrogen		Progesterone		Testosterone		BAT	
	r	p	r	p	r	p	r	p
GRe	0.060	0,715	0,002	0,993	-0,086	0,601	-0.209	0.229
OC	-0.114	0,491	-0,194	0,238	-0,268	0,099	-0.387	0.022*
ITG	0.064	0,733	-0.002	0,988	-0,059	0,720	-0.169	0.330
FuG	0.069	0,675	-0,013	0,937	-0,096	0,562	-0.216	0.213
STG	0,014	0.933	-0.069	0,677	-0,170	0,300	-0.267	0.121
MTG	-0,019	0,906	-0.076	0,647	-0,163	0,322	-0.283	0.100
ACC	0.013	0,935	-0,035	0,834	-0,137	0,406	-0.208	0.230
HPC	0.108	0,514	-0,005	0,978	-0,049	0,769	-0.214	0.217
CHPC	0.040	0,809	-0,082	0,618	-0,115	0,487	-0.236	0.173
PHG	-0.060	0,717	-0.171	0,299	-0,226	0,167	-0.315	0.066
Ins	0.032	0.846	-0,042	0,800	-0,128	0,437	-0.203	0.242
AMY	-0.232	0,155	-0.346*	0,031	-0.405*	0,011	-0.443	0.008**
DRN	0.025	0,880	-0,169	0,305	-0,243	0,137	-0.384	0.023*
MRN	-0.102	0,535	-0.180	0,273	-0,284	0,080	-0.334	0.050*

Table 9 Correlation of 5-HT$_{1A}$ BP of both, pre- and postmenopausal women and steroidal hormones by means of Spearman's rank correlation coefficient. BAT – bioavailable testosterone

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). **No region survived Bonferroni correction for multiple comparisons (adjusted p<0.0035)**

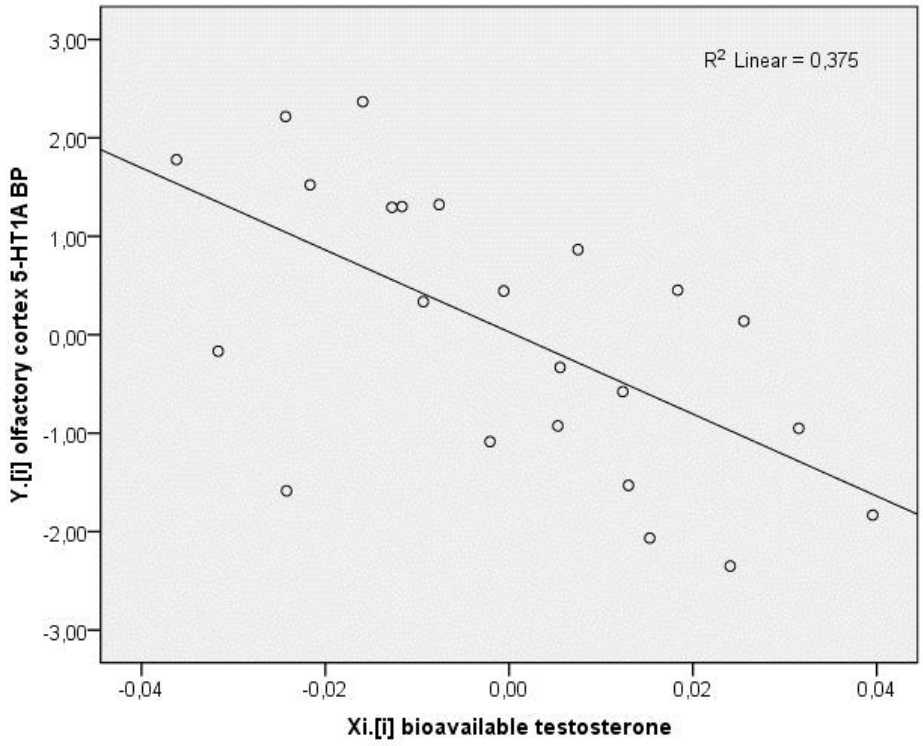
13.5. Correlation of 5-HT_{1A} BP and steroid hormone plasma levels in premenopausal woman

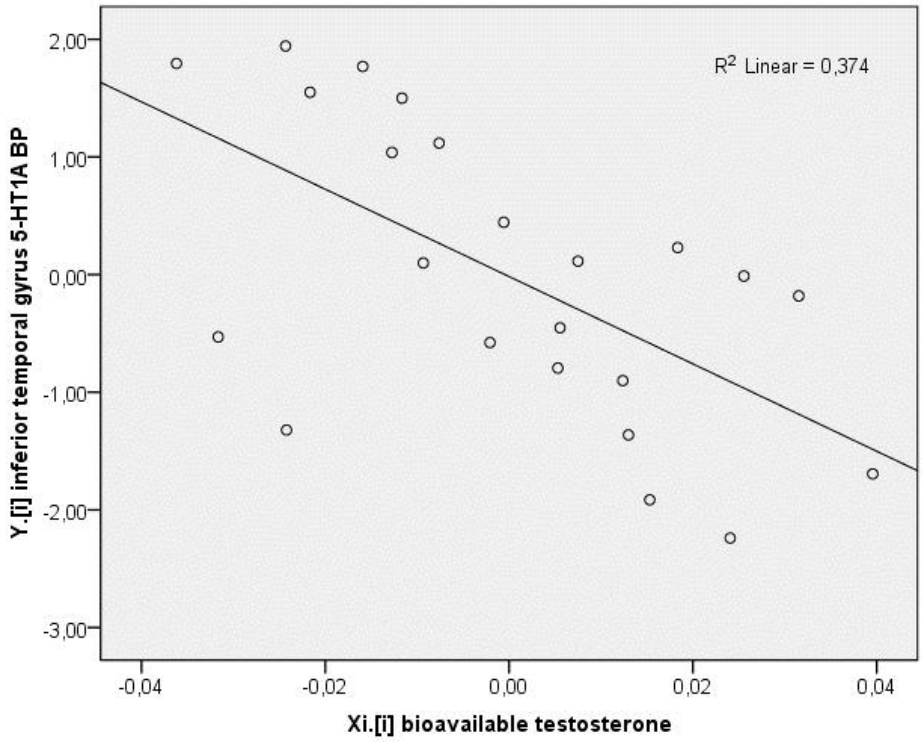
	Estrogen		Progesterone		Testosterone		BAT	
ROI	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
GRe	-0.024	0.934	-0.120	0.682	0.057	0.846	0.237	0.458
OC	-0.077	0.793	-0.141	0.631	0.184	0.530	0.324	0.304
ITG	-0.006	0.983	-0.156	0.595	0.153	0.602	0.300	0.344
FuG	0.007	0.982	-0,116	0.693	0.183	0.531	0.309	0.329
STG	-0.047	0.872	-0.179	0.540	0.066	0.822	0.237	0.459
MTG	0.020	0.946	-0.181	0.536	0.092	0.754	0.280	0.378
ACC	0.021	0.944	-0.147	0.617	0.227	0.436	0.376	0.229
HPC	-0.073	0,805	-0.122	0.678	0.283	0.327	0.296	0.350
CHPC	-0.098	0.738	-0.187	0.522	0.283	0.327	0.318	0.314
PHG	0.033	0.912	-0.128	0.662	0.217	0.456	0.300	0.344
Ins	-0.044	0.882	-0,144	0.624	0.194	0.507	0.312	0.324
AMY	-0.105	0.720	-0.100	0.733	0.042	0,886	0.273	0.391
DRN	0.011	0.970	-0.083	0.777	0.096	0.745	0.158	0.624
MRN	0.093	0.753	0.091	0.758	0.091	0.758	0.107	0.740

Table 10 Correlation of 5-HT_{1A} BP in premenopausal women and steroidal hormones by means of Pearson product moment correlation. **BAT** – bioavailable testosterone; gyrus rectus (**GRe**), olfactory cortex (**OC**), inferior temporal gyrus (**ITG**), fusiform gyrus (**FuG**), superior temporal gyrus (**STG**), middle temporal gyrus (**MTG**), anterior cingulate cortex (**ACC**), hippocampus (**HPC**), caput hippocampi (**CHPC**), parahippocampal gyrus (**PHG**), insula (**Ins**), amygdala (**AMY**), dorsal raphe nucleus (**DRN**), median raphe nucleus (**MRN**)

13.6. Partial regression plots of 5-HT_{1A} BP of postmenopausal subjects and bioavailable testosterone adjusted for estrogen and progesterone

Note:
***Y*$_{.[i]}$** = residuals from regressing Y (the response variable **5-HT_{1A} BP**) against independent variables **estrogen and progesterone** except Xi **bioavailable testosterone;**
***X*$_{i.[i]}$** = residuals from regressing X_i **bioavailable testosterone** against the independent variables **estrogen and progesterone**

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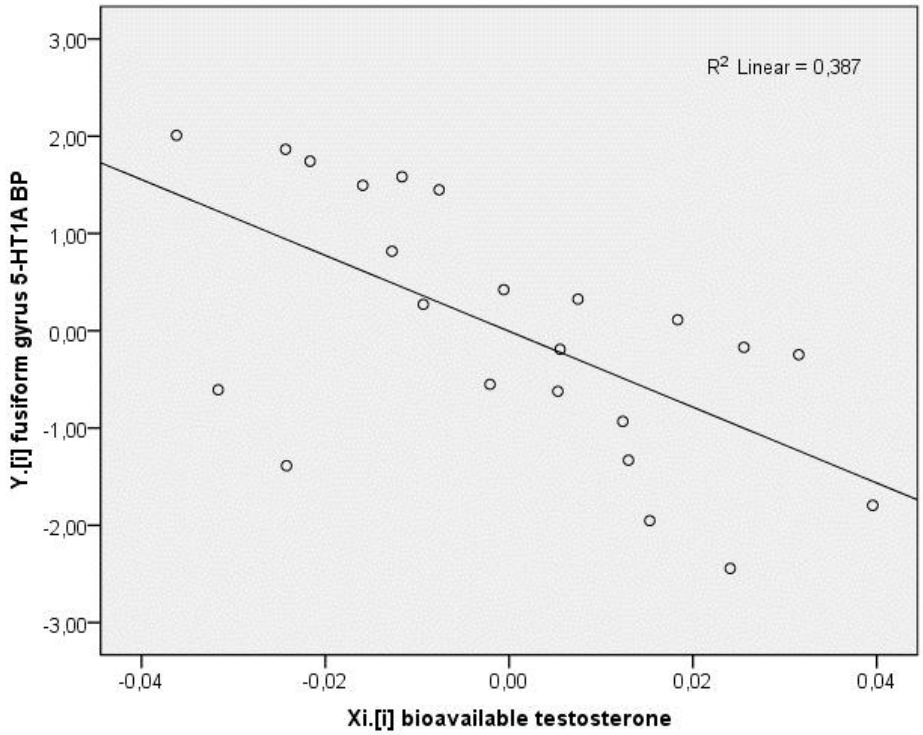
Y.[i] inferior temporal gyrus 5-HT1A BP
2,00
1,00
0,00
-1,00
-2,00
-3,00
-0,04
-0,02
0,00
0,02
0,04
Xi.[i] bioavailable testosterone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Y.[i] fusiform gyrus 5-HT1A BP
3,00
2,00
1,00
0,00
-1,00
-2,00
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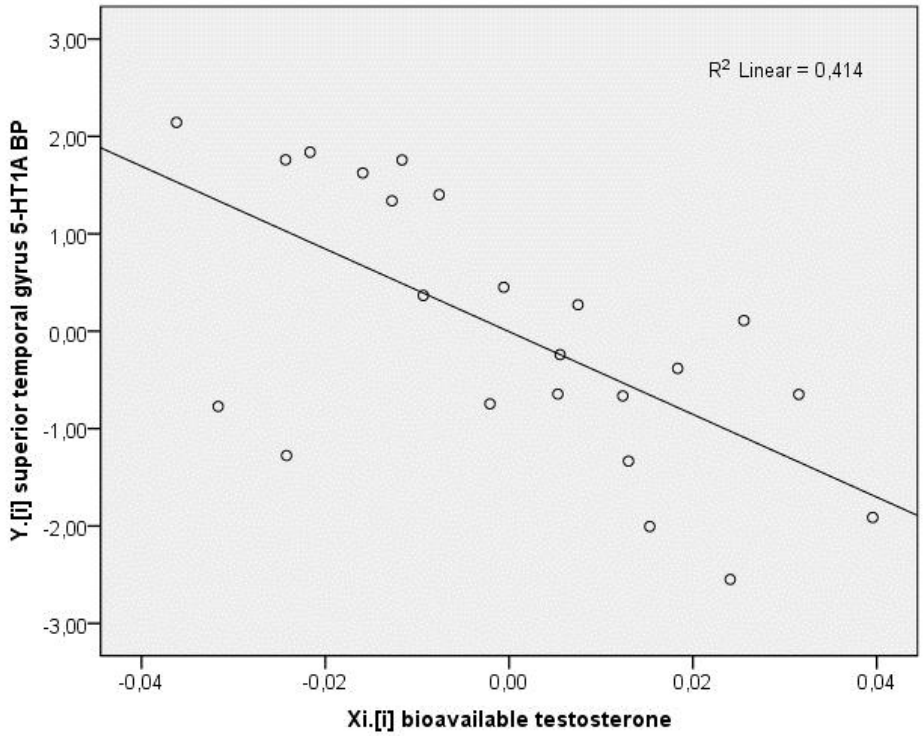
-0,02
0,00
0,02
0,04
Xi.[i] bioavailable testosterone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Y.[i] superior temporal gyrus 5-HT1A BP
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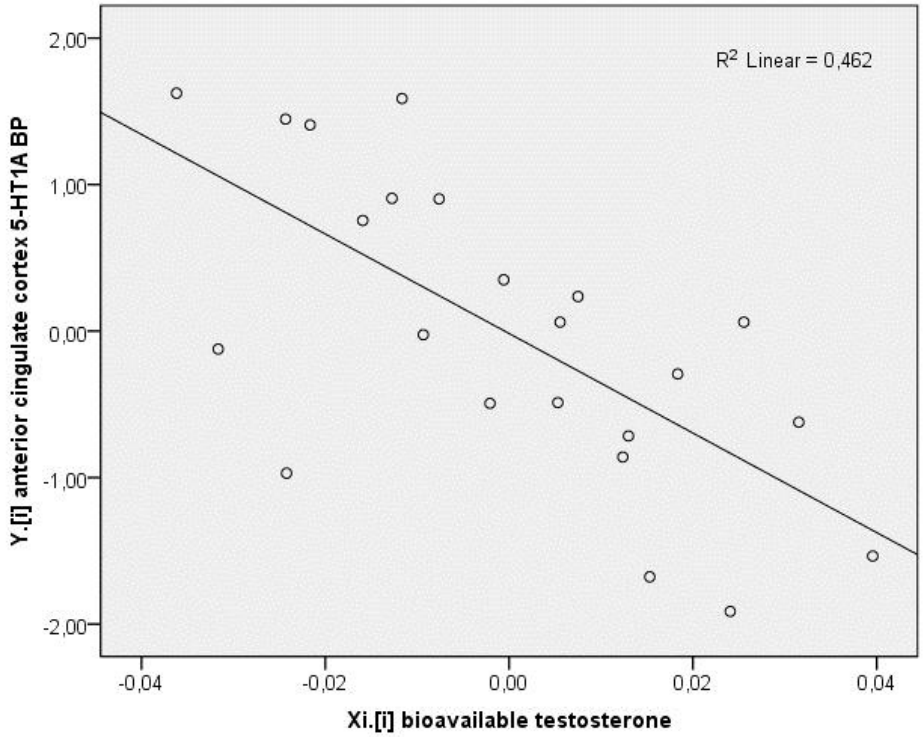
2,00
1,00
0,00
-1,00
-2,00
-3,00
-0,04
-0,02
0,00
0,02
0,04
Xi.[i] bioavailable testosterone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Y.[i] anterior cingulate cortex 5-HT1A BP
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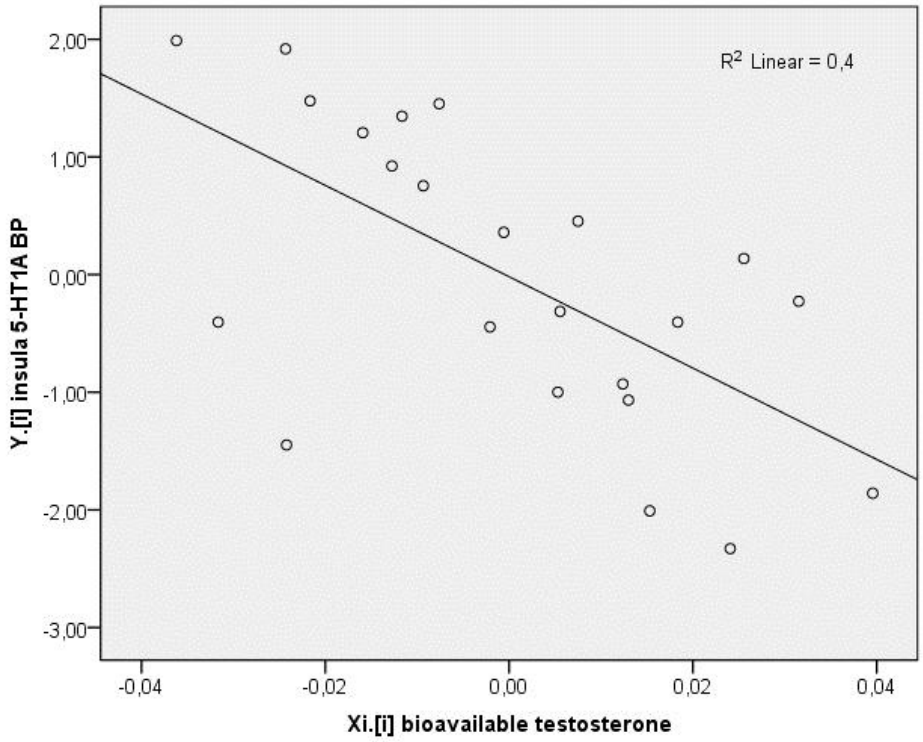
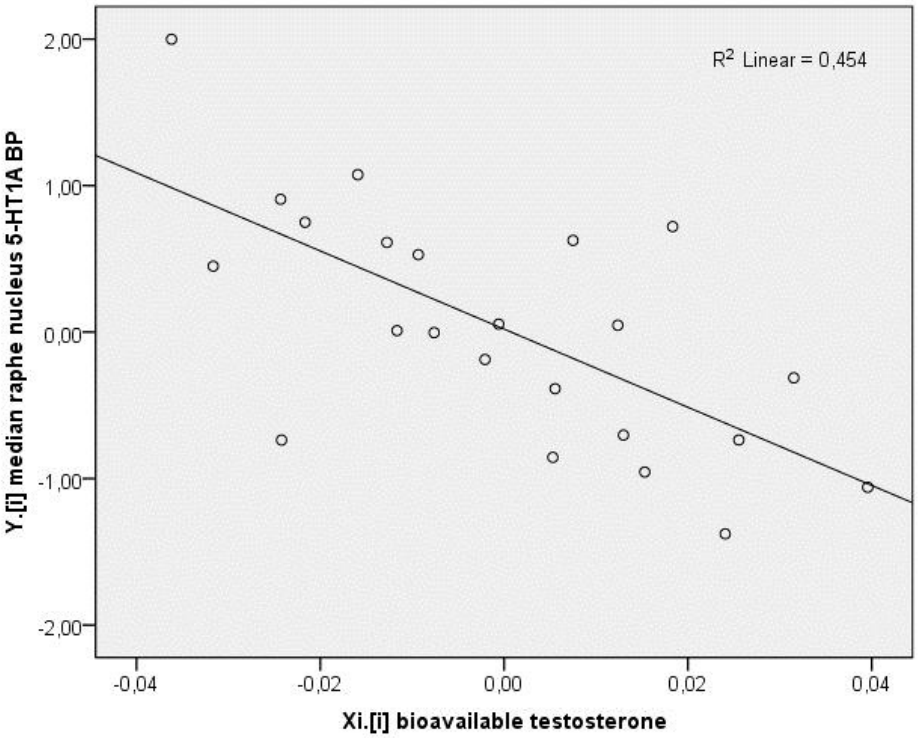
-1,00
-2,00
-0,04
-0,02
0,00
0,02
0,04
Xi.[i] bioavailable testosterone


R² Linear = 0,4
Y.[i] insula 5-HT1A BP
2,00
1,00
0,00
-1,00
-2,00
-3,00
-0,04
-0,02
0,00
0,02
0,04
Xi.[i] bioavailable testosterone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Y.[i] median raphe nucleus 5-HT1A BP
2,00
1,00
0,00
-1,00
-2,00
-0,04
-0,02
0,00
0,02
0,04
Xi.[i] bioavailable testosterone

14. REFERENCES

Adayev T, Ray I, Sondhi R, Sobocki T, Banerjee P (2003) The G protein-coupled 5-HT1A receptor causes suppression of caspase-3 through MAPK and protein kinase Calpha. Biochim Biophys Acta 1640:85-96.

Adell A, Carceller A, Artigas F (1993) In vivo brain dialysis study of the somatodendritic release of serotonin in the Raphe nuclei of the rat: effects of 8-hydroxy-2-(di-n-propylamino)tetralin. J Neurochem 60:1673-1681.

Aghajanian GKS-B, E (2002) Serotonin. In: Neuropsychopharmacology: the Fifth Generation of Progress, pp pp. 15-34: Davis, K.L Charney, D Coyle, J.T. Nemeroff, C.

Agnati LF, Guidolin D, Guescini M, Genedani S, Fuxe K (2010) Understanding wiring and volume transmission. Brain Res Rev 64:137-159.

Allen JA, Yadav PN, Roth BL (2008) Insights into the regulation of 5-HT2A serotonin receptors by scaffolding proteins and kinases. Neuropharmacology 55:961-968.

Alonso J, Angermeyer MC, Bernert S, Bruffaerts R, Brugha TS, Bryson H, de Girolamo G, Graaf R, Demyttenaere K, Gasquet I, Haro JM, Katz SJ, Kessler RC, Kovess V, Lepine JP, Ormel J, Polidori G, Russo LJ, Vilagut G, Almansa J, Arbabzadeh-Bouchez S, Autonell J, Bernal M, Buist-Bouwman MA, Codony M, Domingo-Salvany A, Ferrer M, Joo SS, Martinez-Alonso M, Matschinger H, Mazzi F, Morgan Z, Morosini P, Palacin C, Romera B, Taub N, Vollebergh WA (2004) Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr Scand Suppl 21-27.

Amilhon B, Lepicard E, Renoir T, Mongeau R, Popa D, Poirel O, Miot S, Gras C, Gardier AM, Gallego J, Hamon M, Lanfumey L, Gasnier B, Giros B, El Mestikawy S (2010) VGLUT3 (vesicular glutamate transporter type 3) contribution to the regulation of serotonergic transmission and anxiety. J Neurosci 30:2198-2210.

Amin AH, Crawford TB, Gaddum JH (1954) The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. J Physiol 126:596-618.

Andrade R (2011) Serotonergic regulation of neuronal excitability in the prefrontal cortex. Neuropharmacology 61:382-386.

Andree B, Halldin C, Pike VW, Gunn RN, Olsson H, Farde L (2002) The PET radioligand [carbonyl-(11)C]desmethyl-WAY-100635 binds to 5-HT(1A) receptors and provides a higher radioactive signal than [carbonyl-(11)C]WAY-100635 in the human brain. J Nucl Med 43:292-303.

Ashby CR, Jr., Zhang JY, Edwards E, Wang RY (1994) The induction of serotonin3-like receptor supersensitivity and dopamine receptor subsensitivity in the rat medial prefrontal cortex after the intraventricular administration of the neurotoxin 5,7-dihydroxytryptamine: a microiontophoretic study. Neuroscience 60:453-462.

Azcoitia I, Yague JG, Garcia-Segura LM (2011) Estradiol synthesis within the human brain. Neuroscience 191:139-147.

Azmitia EC (1999) Serotonin neurons, neuroplasticity, and homeostasis of neural tissue. Neuropsychopharmacology 21:33S-45S.

Azmitia EC (2007) Serotonin and brain: evolution, neuroplasticity, and homeostasis. Int Rev Neurobiol 77:31-56.

Backstrom T (1995) Symptoms related to the menopause and sex steroid treatments. Ciba Found Symp 191:171-180; discussion 180-176.

Badawi (1999) Introduction to PET Physics. p http://depts.washington.edu/nucmed/IRL/pet_intro/intro_src/section2.html#2.3: University of Washington, Division of Nuclear Medicine

Badawi RD (1998) Aspects of optimisation and quantification in three-dimensional Positron Emission Tomography., vol. Dissertatoin for the degree of Ph.D. London: University of London, UK.

Baker KG, Halliday GM, Halasz P, Hornung JP, Geffen LB, Cotton RG, Tork I (1991a) Cytoarchitecture of serotonin-synthesizing neurons in the pontine tegmentum of the human brain. Synapse 7:301-320.

Baker KG, Halliday GM, Hornung JP, Geffen LB, Cotton RG, Tork I (1991b) Distribution, morphology and number of monoamine-synthesizing and substance P-containing neurons in the human dorsal raphe nucleus. Neuroscience 42:757-775.

Baldwin D, Rudge S (1995) The role of serotonin in depression and anxiety. Int Clin Psychopharmacol 9 Suppl 4:41-45.

Barnes NM, Hales TG, Lummis SC, Peters JA (2009) The 5-HT3 receptor--the relationship between structure and function. Neuropharmacology 56:273-284.

Benmansour S, Piotrowski JP, Altamirano AV, Frazer A (2009) Impact of ovarian hormones on the modulation of the serotonin transporter by fluvoxamine. Neuropsychopharmacology 34:555-564.

Berek JS, Novak E (2007) Berek & Novak's gynecology. Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Berg JM, Tymoczko JL, Stryer L (2002) Biochemistry. New York: W. H. Freeman and Co. ; [Basingstoke : Palgrave] [distributor], 2001.

Bethea CL, Lu NZ, Gundlah C, Streicher JM (2002a) Diverse actions of ovarian steroids in the serotonin neural system. Front Neuroendocrinol 23:41-100.

Bethea CL, Mirkes SJ, Shively CA, Adams MR (2000) Steroid regulation of tryptophan hydroxylase protein in the dorsal raphe of macaques. Biol Psychiatry 47:562-576.

Bethea CL, Mirkes SJ, Su A, Michelson D (2002b) Effects of oral estrogen, raloxifene and arzoxifene on gene expression in serotonin neurons of macaques. Psychoneuroendocrinology 27:431-445.

Bethea CL, Pecins-Thompson M, Schutzer WE, Gundlah C, Lu ZN (1998) Ovarian steroids and serotonin neural function. Mol Neurobiol 18:87-123.

Bezdickova M, Molikova R, Bebarova L, Kolar Z (2007) Distribution of nuclear receptors for steroid hormones in the human brain: a preliminary study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 151:69-71.

Birzniece V, Johansson IM, Wang MD, Seckl JR, Backstrom T, Olsson T (2001) Serotonin 5-HT(1A) receptor mRNA expression in dorsal hippocampus and raphe nuclei after gonadal hormone manipulation in female rats. Neuroendocrinology 74:135-142.

Blier P, Ward NM (2003) Is there a role for 5-HT1A agonists in the treatment of depression? Biol Psychiatry 53:193-203.

Brinton RD, Thompson RF, Foy MR, Baudry M, Wang J, Finch CE, Morgan TE, Pike CJ, Mack WJ, Stanczyk FZ, Nilsen J (2008) Progesterone receptors: form and function in brain. Front Neuroendocrinol 29:313-339.

Brodie BB, Pletscher A, Shore PA (1955) Evidence that serotonin has a role in brain function. Science 122:968.

Burnet PW, Eastwood SL, Harrison PJ (1997) [3H]WAY-100635 for 5-HT1A receptor autoradiography in human brain: a comparison with [3H]8-OH-DPAT and

demonstration of increased binding in the frontal cortex in schizophrenia. Neurochem Int 30:565-574.

Charnay Y, Leger L (2010) Brain serotonergic circuitries. Dialogues Clin Neurosci 12:471-487.

Cherry SR (2006) The 2006 Henry N. Wagner Lecture: Of mice and men (and positrons)--advances in PET imaging technology. J Nucl Med 47:1735-1745.

Cidis Meltzer C, Drevets WC, Price JC, Mathis CA, Lopresti B, Greer PJ, Villemagne VL, Holt D, Mason NS, Houck PR, Reynolds CF, 3rd, DeKosky ST (2001) Gender-specific aging effects on the serotonin 1A receptor. Brain Res 895:9-17.

Clarke WP, Maayani S (1990) Estrogen effects on 5-HT1A receptors in hippocampal membranes from ovariectomized rats: functional and binding studies. Brain Res 518:287-291.

Clarke WP, Yocca FD, Maayani S (1996) Lack of 5-hydroxytryptamine1A-mediated inhibition of adenylyl cyclase in dorsal raphe of male and female rats. J Pharmacol Exp Ther 277:1259-1266.

Cooke B, Hegstrom CD, Villeneuve LS, Breedlove SM (1998) Sexual differentiation of the vertebrate brain: principles and mechanisms. Front Neuroendocrinol 19:323-362.

Costes N, Merlet I, Ostrowsky K, Faillenot I, Lavenne F, Zimmer L, Ryvlin P, Le Bars D (2005) A 18F-MPPF PET normative database of 5-HT1A receptor binding in men and women over aging. J Nucl Med 46:1980-1989.

Cowen DS, Sowers RS, Manning DR (1996) Activation of a mitogen-activated protein kinase (ERK2) by the 5-hydroxytryptamine1A receptor is sensitive not only to inhibitors of phosphatidylinositol 3-kinase, but to an inhibitor of phosphatidylcholine hydrolysis. J Biol Chem 271:22297-22300.

Crane JW, Shimizu K, Carrasco GA, Garcia F, Jia C, Sullivan NR, D'Souza DN, Zhang Y, Van de Kar LD, Muma NA, Battaglia G (2007) 5-HT1A receptors mediate (+)8-OH-DPAT-stimulation of extracellular signal-regulated kinase (MAP kinase) in vivo in rat hypothalamus: time dependence and regional differences. Brain Res 1183:51-59.

Czajka CP, Londry KL (2006) Anaerobic biotransformation of estrogens. Sci Total Environ 367:932-941.

Dahlman-Wright K, Cavailles V, Fuqua SA, Jordan VC, Katzenellenbogen JA, Korach KS, Maggi A, Muramatsu M, Parker MG, Gustafsson JA (2006) International Union of Pharmacology. LXIV. Estrogen receptors. Pharmacol Rev 58:773-781.

Dalgliesh CE, Toh CC, Work TS (1953) Fractionation of the smooth muscle stimulants present in extracts of gastro-intestinal tract. Identification of 5-hydroxytryptamine and its distinction from substance P. J Physiol 120:298-310.

de Almeida J, Mengod G (2008) Serotonin 1A receptors in human and monkey prefrontal cortex are mainly expressed in pyramidal neurons and in a GABAergic interneuron subpopulation: implications for schizophrenia and its treatment. J Neurochem 107:488-496.

De Vivo M, Maayani S (1986) Characterization of the 5-hydroxytryptamine1a receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. J Pharmacol Exp Ther 238:248-253.

Della Rocca GJ, Mukhin YV, Garnovskaya MN, Daaka Y, Clark GJ, Luttrell LM, Lefkowitz RJ, Raymond JR (1999) Serotonin 5-HT1A receptor-mediated Erk activation requires calcium/calmodulin-dependent receptor endocytosis. J Biol Chem 274:4749-4753.

Descarries L, Beaudet A, Watkins KC (1975) Serotonin nerve terminals in adult rat neocortex. Brain Res 100:563-588.

Descarries L, Mechawar N (2000) Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system. Prog Brain Res 125:27-47.

DonCarlos LL, Sarkey S, Lorenz B, Azcoitia I, Garcia-Ovejero D, Huppenbauer C, Garcia-Segura LM (2006) Novel cellular phenotypes and subcellular sites for androgen action in the forebrain. Neuroscience 138:801-807.

Donner N, Handa RJ (2009) Estrogen receptor beta regulates the expression of tryptophan-hydroxylase 2 mRNA within serotonergic neurons of the rat dorsal raphe nuclei. Neuroscience 163:705-718.

Drevets WC, Frank E, Price JC, Kupfer DJ, Holt D, Greer PJ, Huang Y, Gautier C, Mathis C (1999) PET imaging of serotonin 1A receptor binding in depression. Biol Psychiatry 46:1375-1387.

Drevets WC, Thase ME, Moses-Kolko EL, Price J, Frank E, Kupfer DJ, Mathis C (2007) Serotonin-1A receptor imaging in recurrent depression: replication and literature review. Nucl Med Biol 34:865-877.

Duncan J, Seitz RJ, Kolodny J, Bor D, Herzog H, Ahmed A, Newell FN, Emslie H (2000) A neural basis for general intelligence. Science 289:457-460.

Edinger KL, Frye CA (2005) Testosterone's anti-anxiety and analgesic effects may be due in part to actions of its 5alpha-reduced metabolites in the hippocampus. Psychoneuroendocrinology 30:418-430.

Erritzoe D, Frokjaer VG, Haahr MT, Kalbitzer J, Svarer C, Holst KK, Hansen DL, Jernigan TL, Lehel S, Knudsen GM (2010) Cerebral serotonin transporter binding is inversely related to body mass index. Neuroimage 52:284-289.

Erspamer V (1940) Pharmakologische Studien über Enteramin: Über die Wirkung von Acetonextrakten der Kaninchenmagenschleimhaut auf den Blutdruck und auf isolierte überlebende Organe. Arch Exp Path Pharmakol 196.

Erspamer V, Asero B (1952) Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature 169:800-801.

Erspamer V, Boretti G (1950) Identification of enteramine and enteramine-related substances in extracts of posterior salivary glands of Octopus vulgaris by paper chromatography. Experientia 6:348-349.

Feng Y, Weijdegard B, Wang T, Egecioglu E, Fernandez-Rodriguez J, Huhtaniemi I, Stener-Victorin E, Billig H, Shao R (2010) Spatiotemporal expression of androgen receptors in the female rat brain during the oestrous cycle and the impact of exogenous androgen administration: a comparison with gonadally intact males. Mol Cell Endocrinol 321:161-174.

Fernandez-Guasti A, Kruijver FP, Fodor M, Swaab DF (2000) Sex differences in the distribution of androgen receptors in the human hypothalamus. J Comp Neurol 425:422-435.

Fernandez-Guasti A, Swaab D, Rodriguez-Manzo G (2003) Sexual behavior reduces hypothalamic androgen receptor immunoreactivity. Psychoneuroendocrinology 28:501-512.

Flugge G, Kramer M, Rensing S, Fuchs E (1998) 5HT1A-receptors and behaviour under chronic stress: selective counteraction by testosterone. Eur J Neurosci 10:2685-2693.

Flugge G, Pfender D, Rudolph S, Jarry H, Fuchs E (1999) 5HT1A-receptor binding in the brain of cyclic and ovariectomized female rats. J Neuroendocrinol 11:243-249.

Frankle WG, Slifstein M, Talbot PS, Laruelle M (2005) Neuroreceptor imaging in psychiatry: theory and applications. Int Rev Neurobiol 67:385-440.

Frye CA, Rhodes ME, Rosellini R, Svare B (2002) The nucleus accumbens as a site of action for rewarding properties of testosterone and its 5alpha-reduced metabolites. Pharmacol Biochem Behav 74:119-127.

Gaddum JH, Picarelli ZP (1957) Two kinds of tryptamine receptor. Br J Pharmacol Chemother 12:323-328.

Gershon MD, Takaki M, Tamir H, Branchek T (1985) The enteric neural receptor for 5-hydroxytryptamine. Experientia 41:863-868.

Giangrande PH, McDonnell DP (1999) The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by a single gene. Recent Prog Horm Res 54:291-313; discussion 313-294.

Gjedde A, Wong DF, Rosa-Neto P, Cumming P (2005) Mapping neuroreceptors at work: on the definition and interpretation of binding potentials after 20 years of progress. Int Rev Neurobiol 63:1-20.

Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, Zhou M, Okawa Y, Callado LF, Milligan G, Gingrich JA, Filizola M, Meana JJ, Sealfon SC (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 452:93-97.

Gonzalez-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R, Lira A, Bradley-Moore M, Ge Y, Zhou Q, Sealfon SC, Gingrich JA (2007) Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. Neuron 53:439-452.

Grassi S, Frondaroli A, Di Mauro M, Pettorossi VE (2010) Influence of testosterone on synaptic transmission in the rat medial vestibular nuclei: estrogenic and androgenic effects. Neuroscience 171:666-676.

Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P, Chambon P (1986) Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature 320:134-139.

Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, Shine J (1986) Sequence and expression of human estrogen receptor complementary DNA. Science 231:1150-1154.

Gu Z, Jiang Q, Yan Z (2007) RGS4 modulates serotonin signaling in prefrontal cortex and links to serotonin dysfunction in a rat model of schizophrenia. Mol Pharmacol 71:1030-1039.

Gundlah C, Alves SE, Clark JA, Pai LY, Schaeffer JM, Rohrer SP (2005) Estrogen receptor-beta regulates tryptophan hydroxylase-1 expression in the murine midbrain raphe. Biol Psychiatry 57:938-942.

Gundlah C, Lu NZ, Bethea CL (2002) Ovarian steroid regulation of monoamine oxidase-A and -B mRNAs in the macaque dorsal raphe and hypothalamic nuclei. Psychopharmacology (Berl) 160:271-282.

Gundlah C, Pecins-Thompson M, Schutzer WE, Bethea CL (1999) Ovarian steroid effects on serotonin 1A, 2A and 2C receptor mRNA in macaque hypothalamus. Brain Res Mol Brain Res 63:325-339.

Gutknecht L, Kriegebaum C, Waider J, Schmitt A, Lesch KP (2009) Spatio-temporal expression of tryptophan hydroxylase isoforms in murine and human brain: convergent data from Tph2 knockout mice. Eur Neuropsychopharmacol 19:266-282.

Haddjeri N, Lavoie N, Blier P (2004) Electrophysiological evidence for the tonic activation of 5-HT(1A) autoreceptors in the rat dorsal raphe nucleus. Neuropsychopharmacology 29:1800-1806.

Haddjeri N, Ortemann C, de Montigny C, Blier P (1999) Effect of sustained administration of the 5-HT1A receptor agonist flesinoxan on rat 5-HT neurotransmission. Eur Neuropsychopharmacol 9:427-440.

Hall H, Lundkvist C, Halldin C, Farde L, Pike VW, McCarron JA, Fletcher A, Cliffe IA, Barf T, Wikstrom H, Sedvall G (1997) Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]way-100635. Brain Res 745:96-108.

Handa RJ, Pak TR, Kudwa AE, Lund TD, Hinds L (2008) An alternate pathway for androgen regulation of brain function: activation of estrogen receptor beta by the metabolite of dihydrotestosterone, 5alpha-androstane-3beta,17beta-diol. Horm Behav 53:741-752.

Heiss WD, Herholz K (2006) Brain receptor imaging. J Nucl Med 47:302-312.

Henderson JA, Bethea CL (2008) Differential effects of ovarian steroids and raloxifene on serotonin 1A and 2C receptor protein expression in macaques. Endocrine 33:285-293.

Hildebrandt T, Alfano L, Tricamo M, Pfaff DW (2010) Conceptualizing the role of estrogens and serotonin in the development and maintenance of bulimia nervosa. Clin Psychol Rev 30:655-668.

Hirvonen J, Karlsson H, Kajander J, Lepola A, Markkula J, Rasi-Hakala H, Nagren K, Salminen JK, Hietala J (2008) Decreased brain serotonin 5-HT1A receptor availability in medication-naive patients with **major depressive disorde**r: an in-vivo imaging study using PET and [carbonyl-11C]WAY-100635. Int J Neuropsychopharmacol 11:465-476.

Hornung JP (2003) The human raphe nuclei and the serotonergic system. J Chem Neuroanat 26:331-343.

Ichise M, Meyer JH, Yonekura Y (2001) An introduction to PET and SPECT neuroreceptor quantification models. J Nucl Med 42:755-763.

Idkowiak-Baldys J, Baldys A, Raymond JR, Hannun YA (2009) Sustained receptor stimulation leads to sequestration of recycling endosomes in a classical protein kinase C- and phospholipase D-dependent manner. J Biol Chem 284:22322-22331.

Innis RB, Aghajanian GK (1987) Pertussis toxin blocks 5-HT1A and GABAB receptor-mediated inhibition of serotonergic neurons. Eur J Pharmacol 143:195-204.

Jackson A, Etgen AM (2001) Estrogen modulates 5-HT(1A) agonist inhibition of lordosis behavior but not binding of [(3)H]-8-OH-DPAT. Pharmacol Biochem Behav 68:221-227.

Jacobs BL, Azmitia EC (1992) Structure and function of the brain serotonin system. Physiol Rev 72:165-229.

Jacobsen JP, Siesser WB, Sachs BD, Peterson S, Cools MJ, Setola V, Folgering JH, Flik G, Caron MG (2011) Deficient serotonin neurotransmission and depression-like serotonin biomarker alterations in tryptophan hydroxylase 2 (Tph2) loss-of-function mice. Mol Psychiatry.

Jovanovic H, Cerin A, Karlsson P, Lundberg J, Halldin C, Nordstrom AL (**2006**) A PET study of 5-HT1A receptors at different phases of the menstrual cycle in women with premenstrual dysphoria. Psychiatry Res 148:185-193.

Jovanovic H, Karlsson P, Cerin A, Halldin C, Nordstrom AL (2009) 5-HT(1A) receptor and 5-HTT binding during the menstrual cycle in healthy women examined with [(11)C] WAY100635 and [(11)C] MADAM PET. Psychiatry Res 172:31-37.

Kasamo K, Suzuki T, Tada K, Ueda N, Matsuda E, Ishikawa K, Kojima T (2001) Endogenous 5-HT tonically inhibits spontaneous firing activity of dorsal hippocampus CA1 pyramidal neurons through stimulation of 5-HT(1A) receptors in quiet awake **rats**: in vivo electrophysiological evidence. Neuropsychopharmacology 24:141-151.

Kasper S (2001) Depression and anxiety--separate or continuum? World J Biol Psychiatry 2:162-163.

Kasper S, Tauscher J, Kufferle B, Barnas C, Pezawas L, Quiner S (1999) Dopamine- and serotonin-receptors in schizophrenia: results of imaging-studies and implications for pharmacotherapy in schizophrenia. Eur Arch Psychiatry Clin Neurosci 249 Suppl 4:83-89.

Kawata M (1995) Roles of steroid hormones and their receptors in structural organization in the nervous system. Neurosci Res 24:1-46.

Kriegebaum C, Gutknecht L, Schmitt A, Lesch KP, Reif A (2010) [Serotonin now: Part 1. Neurobiology and developmental genetics]. Fortschr Neurol Psychiatr 78:319-331.

Kroll TM, A.; Elmenhorst, D.; Bauer A. (2011) [18F]altanserin PET in pre- and postmenopausal women is not influenced by physiological estradiol, progesterone, and prolactin levels. In: XXVth International Symposium on Cerebral Blood Flow, Metabolism, and Function & Xth International Conference on Quantification of Brain Function with PET Barcelona, Spain, May 25-28, 2011: Institute of Neurosciences and Medicine, Jülich Research Center, Jülich, Germany.

Kugaya A, Epperson CN, Zoghbi S, van Dyck CH, Hou Y, Fujita M, Staley JK, Garg PK, Seibyl JP, Innis RB (2003) Increase in prefrontal cortex serotonin 2A receptors following estrogen treatment in postmenopausal women. Am J Psychiatry 160:1522-1524.

Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA (1996) Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 93:5925-5930.

Lakoski J (1988) Estrogen-induced modulation of serotonin 5HT1A mediated responses in the dorsal raphe nucleus (DRN). Pharmacologist 30:Abstract No. 126.

Lammertsma AA, Hume SP (1996) Simplified reference tissue model for PET receptor studies. Neuroimage 4:153-158.

Landry M, Di Paolo T (2003) Effect of chronic estradiol, tamoxifen or raloxifene treatment on serotonin 5-HT1A receptor. Brain Res Mol Brain Res 112:82-89.

Lanzenberger R, Mitterhauser M, Kranz GS, Spindelegger C, Wadsak W, Stein P, Moser U, Savli M, Kletter K, Kasper S (2011) Progesterone level predicts serotonin-1a receptor binding in the male human brain. Neuroendocrinology 94:84-88.

Lanzenberger R, Spindelegger C, Holik A, Mien LK, Mitterhauser M, Wadsak W (2005) Decreased serotonin-1A receptor binding in social anxiety disorder. Hawaii, USA: American College of Neuropsychopharmacology (ACNP).

Lanzenberger R, Wadsak W, Spindelegger C, Mitterhauser M, Akimova E, Mien LK, Fink M, Moser U, Savli M, Kranz GS, Hahn A, Kletter K, Kasper S (2010) Cortisol plasma levels in social anxiety disorder patients correlate with serotonin-1A receptor binding in limbic brain regions. Int J Neuropsychopharmacol 13:1129-1143.

Lanzenberger RR, Mitterhauser M, Spindelegger C, Wadsak W, Klein N, Mien LK, Holik A, Attarbaschi T, Mossaheb N, Sacher J, Geiss-Granadia T, Kletter K, Kasper S, Tauscher J

(2007) Reduced serotonin-1A receptor binding in social anxiety disorder. Biol Psychiatry 61:1081-1089.

Le Saux M, Di Paolo T (2005) Changes in 5-HT1A receptor binding and G-protein activation in the rat brain after estrogen treatment: comparison with tamoxifen and raloxifene. J Psychiatry Neurosci 30:110-117.

Leonhardt SA, Boonyaratanakornkit V, Edwards DP (2003) Progesterone receptor transcription and non-transcription signaling mechanisms. Steroids 68:761-770.

Lephart ED (1996) A review of brain aromatase cytochrome P450. Brain Res Brain Res Rev 22:1-26.

Lephart ED, Lund TD, Horvath TL (2001) Brain androgen and progesterone metabolizing enzymes: biosynthesis, distribution and function. Brain Res Brain Res Rev 37:25-37.

Levine JE, Chappell PE, Schneider JS, Sleiter NC, Szabo M (2001) Progesterone receptors as neuroendocrine integrators. Front Neuroendocrinol 22:69-106.

Lu NZ, Bethea CL (2002) Ovarian steroid regulation of 5-HT1A receptor binding and G protein activation in female monkeys. Neuropsychopharmacology 27:12-24.

Lu NZ, Shlaes TA, Gundlah C, Dziennis SE, Lyle RE, Bethea CL (1999) Ovarian steroid action on tryptophan hydroxylase protein and serotonin compared to localization of ovarian steroid receptors in midbrain of guinea pigs. Endocrine 11:257-267.

Lu NZ, Wardell SE, Burnstein KL, Defranco D, Fuller PJ, Giguere V, Hochberg RB, McKay L, Renoir JM, Weigel NL, Wilson EM, McDonnell DP, Cidlowski JA (2006) International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. Pharmacol Rev 58:782-797.

Luine VN, McEwen BS (1983) Sex differences in cholinergic enzymes of diagonal band nuclei in the rat preoptic area. Neuroendocrinology 36:475-482.

Luine VN, Rhodes JC (1983) Gonadal hormone regulation of MAO and other enzymes in hypothalamic areas. Neuroendocrinology 36:235-241.

Lundberg J, Borg J, Halldin C, Farde L (2007) A PET study on regional coexpression of 5-HT1A receptors and 5-HTT in the human brain. Psychopharmacology (Berl) 195:425-433.

Lynch CS, Story AJ (2000) Dihydrotestosterone and estrogen regulation of rat brain androgen-receptor immunoreactivity. Physiol Behav 69:445-453.

Mangal RK, Wiehle RD, Poindexter AN, 3rd, Weigel NL (1997) Differential expression of uterine progesterone receptor forms A and B during the menstrual cycle. J Steroid Biochem Mol Biol 63:195-202.

Mannoury la Cour C, El Mestikawy S, Hanoun N, Hamon M, Lanfumey L (2006) Regional differences in the coupling of 5-hydroxytryptamine-1A receptors to G proteins in the rat brain. Mol Pharmacol 70:1013-1021.

Martinez D, Slifstein M, Gil R, Hwang DR, Huang Y, Perez A, Frankle WG, Laruelle M, Krystal J, Abi-Dargham A (2009) Positron emission tomography imaging of the serotonin transporter and 5-HT(1A) receptor in alcohol dependence. Biol Psychiatry 65:175-180.

Matzel LD, Kolata S (2010) Selective attention, working memory, and animal intelligence. Neurosci Biobehav Rev 34:23-30.

Melcangi RC, Panzica G, Garcia-Segura LM (2011) Neuroactive steroids: focus on human brain. Neuroscience 191:1-5.

Meltzer CC, Price JC, Mathis CA, Butters MA, Ziolko SK, Moses-Kolko E, Mazumdar S, Mulsant BH, Houck PR, Lopresti BJ, Weissfeld LA, Reynolds CF (2004) Serotonin 1A receptor

binding and treatment response in late-life depression. Neuropsychopharmacology 29:2258-2265.

Menard CS, Harlan RE (1993) Up-regulation of androgen receptor immunoreactivity in the rat brain by androgenic-anabolic steroids. Brain Res 622:226-236.

Meyer JH, Gunn RN, Myers R, Grasby PM (1999) Assessment of spatial normalization of PET ligand images using ligand-specific templates. Neuroimage 9:545-553.

Michaelis L MM (1913) Die Kinetik der Invertinwirkung. Biochem Z 49:1333.

Mikolajczyk K, Szabatin M, Rudnicki P, Grodzki M, Burger C (1998) A JAVA environment for medical image data analysis: initial application for brain PET quantitation. Med Inform (Lond) 23:207-214.

Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ (1984) A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. Ann Neurol 15:217-227.

Mize AA, RH (2000) 17b-Estradiol rapidly decreases 5-HT1A receptor function in rat hippocampus in vitro: Role of PKA and PKC. Soc Neurosci 26:394 (Abstract No. 144.313).

Mize AL, Alper RH (2000) Acute and long-term effects of 17beta-estradiol on G(i/o) coupled neurotransmitter receptor function in the female rat brain as assessed by agonist-stimulated [35S]GTPgammaS binding. Brain Res 859:326-333.

Mize AL, Alper RH (2002) Rapid uncoupling of serotonin-1A receptors in rat hippocampus by 17beta-estradiol in vitro requires protein kinases A and C. Neuroendocrinology 76:339-347.

Mize AL, Poisner AM, Alper RH (2001) Estrogens act in rat hippocampus and frontal cortex to produce rapid, receptor-mediated decreases in serotonin 5-HT(1A) receptor function. Neuroendocrinology 73:166-174.

Mize AL, Young LJ, Alper RH (2003) Uncoupling of 5-HT1A receptors in the brain by estrogens: regional variations in antagonism by ICI 182,780. Neuropharmacology 44:584-591.

Moller M, Jakobsen S, Gjedde A (2007) Parametric and regional maps of free serotonin 5HT1A receptor sites in human brain as function of age in healthy humans. Neuropsychopharmacology 32:1707-1714.

Moller M, Rodell A, Gjedde A (2009) Parametric mapping of 5HT1A receptor sites in the human brain with the Hypotime method: theory and normal values. J Nucl Med 50:1229-1236.

Moser U, Wadsak W, Spindelegger C, Mitterhauser M, Mien LK, Bieglmayer C, Kletter K, Kasper S, Lanzenberger R (2010) Hypothalamic serotonin-1A receptor binding measured by PET predicts the plasma level of dehydroepiandrosterone sulfate in healthy women. Neurosci Lett 476:161-165.

Moses-Kolko EL, Berga SL, Greer PJ, Smith G, Cidis Meltzer C, Drevets WC (2003) Widespread increases of cortical serotonin type 2A receptor availability after hormone therapy in euthymic postmenopausal women. Fertil Steril 80:554-559.

Moses-Kolko EL, Price JC, Shah N, Berga S, Sereika SM, Fisher PM, Coleman R, Becker C, Mason NS, Loucks T, Meltzer CC (2011) Age, Sex, and Reproductive Hormone Effects on Brain Serotonin-1A and Serotonin-2A Receptor Binding in a Healthy Population. Neuropsychopharmacology.

Moses EL, Drevets WC, Smith G, Mathis CA, Kalro BN, Butters MA, Leondires MP, Greer PJ, Lopresti B, Loucks TL, Berga SL (2000) Effects of estradiol and progesterone

administration on human serotonin 2A receptor binding: a PET study. Biol Psychiatry 48:854-860.

Mukai H, Takata N, Ishii HT, Tanabe N, Hojo Y, Furukawa A, Kimoto T, Kawato S (2006) Hippocampal synthesis of estrogens and androgens which are paracrine modulators of synaptic plasticity: synaptocrinology. Neuroscience 138:757-764.

Naess O (1976) Characterization of the androgen receptors in the hypothalamus, preoptic area and brain cortex of the rat. Steroids 27:167-185.

Narr KL, Woods RP, Thompson PM, Szeszko P, Robinson D, Dimtcheva T, Gurbani M, Toga AW, Bilder RM (2007) Relationships between IQ and regional cortical gray matter thickness in healthy adults. Cereb Cortex 17:2163-2171.

Neumeister A, Bain E, Nugent AC, Carson RE, Bonne O, Luckenbaugh DA, Eckelman W, Herscovitch P, Charney DS, Drevets WC (2004) Reduced serotonin type 1A receptor binding in panic disorder. J Neurosci 24:589-591.

No authors listed (1996) Research on the menopause in the 1990s. Report of a WHO Scientific Group. In: World Health Organ Tech Rep Ser, vol. 866, pp 1-107.

Ortega-Corona BG, Valencia-Sanchez A, Kubli-Garfias C, Anton-Tay F, Salazar LA, Villarreal JE, Ponce-Monter H (1994) Hypothalamic monoamine oxidase activity in ovariectomized rats after sexual behavior restoration. Arch Med Res 25:337-340.

Osterlund M, Kuiper GG, Gustafsson JA, Hurd YL (1998) Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. Brain Res Mol Brain Res 54:175-180.

Osterlund MK (2010) Underlying mechanisms mediating the antidepressant effects of estrogens. Biochim Biophys Acta 1800:1136-1144.

Osterlund MK, Halldin C, Hurd YL (2000) Effects of chronic 17beta-estradiol treatment on the serotonin 5-HT(1A) receptor mRNA and binding levels in the rat brain. Synapse 35:39-44.

Owens MJ, Nemeroff CB (1998) The serotonin transporter and depression. Depress Anxiety 8 Suppl 1:5-12.

Palchaudhuri M, Flugge G (2005) 5-HT1A receptor expression in pyramidal neurons of cortical and limbic brain regions. Cell Tissue Res 321:159-172.

Panzica GC, Aste N, Viglietti-Panzica C, Ottinger MA (1995) Structural sex differences in the brain: influence of gonadal steroids and behavioral correlates. J Endocrinol Invest 18:232-252.

Parl FF (2000) Estrogens, estrogen receptor and breast cancer. Amsterdam ; Oxford: IOS Press/Ohmsha.

Parsey RV (2010) Serotonin receptor imaging: clinically useful? J Nucl Med 51:1495-1498.

Parsey RV, Ogden RT, Miller JM, Tin A, Hesselgrave N, Goldstein E, Mikhno A, Milak M, Zanderigo F, Sullivan GM, Oquendo MA, Mann JJ (2010) Higher serotonin 1A binding in a second major depression cohort: modeling and reference region considerations. Biol Psychiatry 68:170-178.

Parsey RV, Oquendo MA, Ogden RT, Olvet DM, Simpson N, Huang YY, Van Heertum RL, Arango V, Mann JJ (2006) Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. Biol Psychiatry 59:106-113.

Parsey RV, Oquendo MA, Simpson NR, Ogden RT, Van Heertum R, Arango V, Mann JJ (2002) Effects of **sex, age**, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. Brain Res 954:173-182.

Pecins-Thompson M, Bethea CL (1999) Ovarian steroid regulation of serotonin-1A autoreceptor messenger RNA expression in the dorsal raphe of rhesus macaques. Neuroscience 89:267-277.

Pecins-Thompson MB, CL (1998) Ovarian steroid regulation of 5HT1A autoreceptor messenger ribonucleic acid expression in the dorsal raphe of rhesus macaques. Neuroscience 89:267–277.

Penington NJ, Kelly JS (1990) Serotonin receptor activation reduces calcium current in an acutely dissociated adult central neuron. Neuron 4:751-758.

Penington NJ, Kelly JS, Fox AP (1991) A study of the mechanism of Ca2+ current inhibition produced by serotonin in rat dorsal raphe neurons. J Neurosci 11:3594-3609.

Penington NJ, Kelly JS, Fox AP (1993) Whole-cell recordings of inwardly rectifying K+ currents activated by 5-HT1A receptors on dorsal raphe neurones of the adult rat. J Physiol 469:387-405.

Pike VW, McCarron JA, Lammertsma AA, Osman S, Hume SP, Sargent PA, Bench CJ, Cliffe IA, Fletcher A, Grasby PM (1996) Exquisite delineation of 5-HT1A receptors in human brain with PET and [carbonyl-11 C]WAY-100635. Eur J Pharmacol 301:R5-7.

Puy L, MacLusky NJ, Becker L, Karsan N, Trachtenberg J, Brown TJ (1995) Immunocytochemical detection of androgen receptor in human temporal cortex characterization and application of polyclonal androgen receptor antibodies in frozen and paraffin-embedded tissues. J Steroid Biochem Mol Biol 55:197-209.

Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD, Bench CJ, Gunn RN, Cowen P, Grasby PM (2002) A database of [(11)C]WAY-100635 binding to 5-HT(1A) receptors in normal **male** volunteers: normative data and relationship to methodological, demographic, physiological, and behavioral variables. Neuroimage 15:620-632.

Ramirez VD, Zheng J (1996) Membrane sex-steroid receptors in the brain. Front Neuroendocrinol 17:402-439.

Rapport MM, Green AA, Page IH (1948a) Crystalline Serotonin. Science 108:329-330.

Rapport MM, Green AA, Page IH (1948b) Serum vasoconstrictor, serotonin; isolation and characterization. J Biol Chem 176:1243-1251.

Rhee JS, Ishibashi H, Akaike N (1996) Serotonin modulates high-voltage-activated Ca2+ channels in rat ventromedial hypothalamic neurons. Neuropharmacology 35:1093-1100.

Riad M, Zimmer L, Rbah L, Watkins KC, Hamon M, Descarries L (2004) Acute treatment with the antidepressant fluoxetine internalizes 5-HT1A autoreceptors and reduces the in vivo binding of the PET radioligand [18F]MPPF in the nucleus raphe dorsalis of rat. J Neurosci 24:5420-5426.

Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB (2002) Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem 277:5209-5218.

Rossmanith WG, Scherbaum WA (1992) Neuroendocrinology of sex steroids : basic knowledge and clinical implications. Berlin: Walter de Gruyter.

Roth BL (2006) The serotonin receptors : from molecular pharmacology to human therapeutics. Totowa, N.J.: Humana Press.

Saigal N, Pichika R, Easwaramoorthy B, Collins D, Christian BT, Shi B, Narayanan TK, Potkin SG, Mukherjee J (2006) Synthesis and biologic evaluation of a novel serotonin 5-HT1A

receptor radioligand, 18F-labeled mefway, in rodents and imaging by PET in a nonhuman primate. J Nucl Med 47:1697-1706.

Sanchez RL, Reddy AP, Centeno ML, Henderson JA, Bethea CL (2005) A second tryptophan hydroxylase isoform, TPH-2 mRNA, is increased by ovarian steroids in the raphe region of macaques. Brain Res Mol Brain Res 135:194-203.

Santana N, Bortolozzi A, Serrats J, Mengod G, Artigas F (2004) Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. Cereb Cortex 14:1100-1109.

Sarrieau A, Dussaillant M, Agid F, Philibert D, Agid Y, Rostene W (1986) Autoradiographic localization of glucocorticosteroid and progesterone binding sites in the human post-mortem brain. J Steroid Biochem 25:717-721.

Sarrieau A, Mitchell JB, Lal S, Olivier A, Quirion R, Meaney MJ (1990) Androgen binding sites in human temporal cortex. Neuroendocrinology 51:713-716.

Sheng Z, Kawano J, Yanai A, Fujinaga R, Tanaka M, Watanabe Y, Shinoda K (2004) Expression of estrogen receptors (alpha, beta) and androgen receptor in serotonin neurons of the rat and mouse dorsal raphe nuclei; sex and species differences. Neurosci Res 49:185-196.

Siegel GJ AB, Albers RW (1999) Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition. Philadelphia: Lippincott-Raven.

Simon NG, Cologer-Clifford A, Lu SF, McKenna SE, Hu S (1998) Testosterone and its metabolites modulate 5HT1A and 5HT1B agonist effects on intermale aggression. Neurosci Biobehav Rev 23:325-336.

Sinton CM, Fallon SL (1988) Electrophysiological evidence for a functional differentiation between subtypes of the 5-HT1 receptor. Eur J Pharmacol 157:173-181.

Stein P, Savli M, Wadsak W, Mitterhauser M, Fink M, Spindelegger C, Mien LK, Moser U, Dudczak R, Kletter K, Kasper S, Lanzenberger R (2008) The serotonin-1A receptor distribution in healthy men and women measured by PET and [carbonyl-11C]WAY-100635. Eur J Nucl Med Mol Imaging 35:2159-2168.

Stockmeier CA (2003) Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. J Psychiatr Res 37:357-373.

Stuenkel CA (1989) Menopause and estrogen replacement therapy. Psychiatr Clin North Am 12:133-152.

Tauscher J, Verhoeff NP, Christensen BK, Hussey D, Meyer JH, Kecojevic A, Javanmard M, Kasper S, Kapur S (2001) Serotonin 5-HT1A receptor binding potential **declines with age** as measured by [11C]WAY-100635 and PET. Neuropsychopharmacology 24:522-530.

Twarog BM, Page IH (1953) Serotonin content of some mammalian tissues and urine and a method for its determination. Am J Physiol 175:157-161.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15:273-289.

Urban RJ, Dahl KD, Padmanabhan V, Beitins IZ, Veldhuis JD (1991) Specific regulatory actions of dihydrotestosterone and estradiol on the dynamics of FSH secretion and clearance in humans. J Androl 12:27-35.

Varnas K, Halldin C, Hall H (2004) Autoradiographic distribution of serotonin transporters and receptor subtypes in human brain. Hum Brain Mapp 22:246-260.

Wadsak W, Mitterhauser M (2010) Basics and principles of radiopharmaceuticals for PET/CT. Eur J Radiol 73:461-469.

Walther DJ, Bader M (2003) A unique central tryptophan hydroxylase isoform. Biochem Pharmacol 66:1673-1680.

Weihua Z, Andersson S, Cheng G, Simpson ER, Warner M, Gustafsson JA (2003) Update on estrogen signaling. FEBS Lett 546:17-24.

Wilson ME, Westberry JM, Trout AL (2011) Estrogen receptor-alpha gene expression in the cortex: sex differences during development and in adulthood. Horm Behav 59:353-357.

Witte AV, Floel A, Stein P, Savli M, Mien LK, Wadsak W, Spindelegger C, Moser U, Fink M, Hahn A, Mitterhauser M, Kletter K, Kasper S, Lanzenberger R (2009) Aggression is related to frontal serotonin-1A receptor distribution as revealed by PET in healthy subjects. Hum Brain Mapp 30:2558-2570.

Wu Y, Carson RE (2002) Noise reduction in the simplified reference tissue model for neuroreceptor functional imaging. J Cereb Blood Flow Metab 22:1440-1452.

Zhang L, Ma W, Barker JL, Rubinow DR (1999) Sex differences in expression of serotonin receptors (subtypes 1A and 2A) in rat brain: a possible role of testosterone. Neuroscience 94:251-259.

Zimmer L, Riad M, Rbah L, Belkacem-Kahlouli A, Le Bars D, Renaud B, Descarries L (2004) Toward brain imaging of serotonin 5-HT1A autoreceptor internalization. Neuroimage 22:1421-1426.