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# GENDER AND THE USE OF HORMONAL CONTRACEPTION IN WOMEN ARE NOT ASSOCIATED WITH CEREBRAL CORTICAL 5-HT 2A RECEPTOR BINDING

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**Abstract—Gender influences brain function including serotonergic neurotransmission, which may play a role in the well-known gender variations in vulnerability to mood and anxiety disorders. Even though hormonal replacement therapy in menopause is associated with globally increased cerebral 5-$HT_{2A}$ receptor binding it is not clear if gender or use of hormonal contraception exhibits associations with 5-$HT_{2A}$ receptor binding. We found no significant effect of gender on cortical 5-$HT_{2A}$ receptor binding ($P$=0.15, $n$=132). When adjusting for the personality trait neuroticism, known to be positively correlated to frontolimbic 5-$HT_{2A}$ receptor binding and to be more pronounced in women, again, the effect of gender was not significant ($P$=0.42, $n$=127). Also, the use of hormonal contraception ($n$=14) within the group of pre-menopausal women (total $n$=29) was not associated with cortical 5-$HT_{2A}$ receptor binding ($P$=0.31). In conclusion, neither gender, nor the use of hormonal contraception in premenopausal women was associated with cortical 5-$HT_{2A}$ receptor binding.** 

**Key words: 5-HT, PET, altanserin, sex, hormones, estrogen.**

Gender influences brain function including key elements of serotonergic neurotransmission (Cahill, 2006; Cosgrove et al., 2007). Dysfunction of serotonergic neurotransmission is critically involved in the pathophysiology of many neuropsychiatric disorders including mood and anxiety disorders (Naughton et al., 2000; Stockmeier, 2003). The vulnerability to mood and anxiety disorders (Pigott, 1999) is considerably greater in women, e.g. major depression is twice as prevalent in women as in men (Kessler et al., 1993), possibly reflecting gender differences in central serotonergic function. Interestingly, the vulnerability to mood disorders and schizophrenia in women is increased in phases where sex hormone levels fluctuate. Postpartum and postmenopausal women have an increased risk of depression (Pearlstein et al., 1997). Moreover, in the perimenopausal phase, women with no history of depression are at increased risk of developing depression, and this risk is associated with increased variability of estradiol levels (Freeman et al., 2006). Also, in women the incidence of schizophrenia is increased in the years following menopause, whereas in men late onset is very rare (Riecher-Rössler and Häfner, 1993). Suicidal behavior also varies with gender (Lewinsohn et al., 2001; Oquendo and Mann, 2000).

Gender differences may come about partly through gonadal hormones, e.g., estrogen, known to be involved in the sexual differentiation of the brain contributing to differences in functional connectivity (Kilpatrick et al., 2006), brain structure, function, and neurochemistry, see review by Cosgrove et al. (2007). However, specific effects of the sex chromosomes on the cellular level are also likely to play a role (Arnold et al., 2004). Hence gender and the use of sex hormones as in hormonal therapy may critically influence serotonergic neurotransmission.

Specifically, studies in monkey have demonstrated that serotonergic neurons in the dorsal raphe express estrogen receptor beta and progestin receptors, and thus are targets for ovarian steroids estrogen and progesterone (Bethea et al., 2002). Serotonergic neurons originating from the dorsal raphe nuclei project to limbic and cortical structures, practically within the entire brain. Therefore, functional alterations of those neurons can affect cortical parts of the serotonergic system, e.g. the postsynaptic 5-$HT_{2A}$ receptor. Also, estradiol can induce transcription of the 5-$HT_{2A}$ receptor gene through actions on the estrogen receptors of either subtype, alpha or beta as demonstrated in rats (Sumner et al., 2007). In general, estrogen receptor alpha and beta are expressed in a limbic-related pattern. The estrogen alpha subtype dominates in amygdala and hypothalamus and the beta subtype in hippocampus, entorhinal cortex, thalamus, and midbrain. As such, estrogen actions can modulate structures involved in neuropsychiatric disorders (Östlund et al., 2003).

As shown in non-human primates, gonadal hormones may increase serotonergic neurotransmission through downregulation of inhibitory 5-HT 1A autoreceptors in the dorsal raphe nuclei (Henderson and Bethea, 2008), and through decreasing the activation of inhibitory G-protein coupled to 5-HT 1A (Lu and Bethea, 2002). Also, estradiol may increase synthesis of 5-HT and decrease degradation through actions on tryptophan hydroxylase (Bethea et al., 2000) and monoamine oxidase A (Gundlah et al., 2002), respectively. Even though serotonergic tonus may increase in response to estradiol and progesterone expo-

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*Abbreviations:* BMI, body mass index; $BP_P$, binding potential the ratio at equilibrium of specifically bound tracer to that of total parent tracer in plasma; MR, magnetic resonance; NEO-PI-R, NEO Personality Inventory Revised; PET, positron emission tomography; SERT, 5-HT transporter.

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sure, the 5-HT transporter (SERT) protein levels seem to increase predominantly in the axonal fibers and nerve terminals (Lu et al., 2003) which might counterbalance the increased extracellular 5-HT. However, hormone replacement therapy leads to a reduction in SERT mRNA in the dorsal raphe nuclei without a concomitant change SERT protein levels, suggestive of a low turnover of central SERT (Pecins-Thompson et al., 1998).

In human studies some gender differences in the serotonergic neurotransmission have been demonstrated. The synthesis rate of 5-HT has been reported to be 50% larger in men than women (Nishizawa et al., 1997). Further, the 5-HT 1A receptor availability—somewhat reflecting an inhibitory capacity—is lower in men (Arango et al., 1995; Parsey et al., 2002; Jovanovic et al., 2008). There are also indications that 5-HT 1A receptor availability may vary with the menstrual cycle in women with premenstrual dysphoric disorder (Jovanovic et al., 2006). However, this does not seem to be the case in healthy women (Jovanovic et al., 2009). Whether SERT availability differs with gender is not clear. Some observe higher SERT-binding in healthy women with the non-selective Single Photon Emission Computed Tomography (SPECT) tracer 123I-beta-CIT (Staley et al., 2001). Others find a lower SERT binding in women based on imaging with the selective SERT tracer 11 C-MADAM positron emission tomography (PET) in eight women and 10 men (Jovanovic, 2008). Yet, Meyer et al. (2004) report no gender differences with the selective PET-tracer 11 C-DASB. In depressed patients, the literature on gender differences in SERT availability is mixed too (Mann et al., 2000; Ruhé et al., 2009; Staley et al., 2006). Also, the effect of gender on 5-$HT_{2A}$ receptor binding, and possible effects of hormonal therapy are not well elucidated. In an early study of 22 healthy subjects higher 5-$HT_{2A}$ receptor binding was found in men than in women (Biver et al., 1996). Yet, others, including our group, have not observed any gender effects (Adams et al., 2004; Lewis et al., 1999). The effect of hormonal replacement therapy in postmenopausal women has been investigated in two smaller studies. (1) In a study of five females, Moses et al. (2000) reported that cortical 5-$HT_{2A}$ receptor binding increased in response to *combined* estradiol and progesterone therapy, but *not* when administering estradiol alone, and (2) Kugaya et al. (2003) found increased prefrontal, and mainly right-sided, cortical 5-$HT_{2A}$ receptor binding when administering estrogen alone in a study of 10 women. These effects have been shown in menopausal women only, whereas, so far, the effect of hormonal contraception in premenopausal women has not been studied.

Studying sex differences at the neurobiological level is critical to improve the etiological understanding of brain disorders with sex difference in their incidence and/or nature. In addition, when designing studies of serotonergic neurotransmission it is highly important to ensure that the groups studied are representative of the population for which the conclusions drawn will be relevant, and at the same time to control for confounding effects. However, due to the possible effects of endogenous estradiol levels and hormonal therapy on 5-$HT_{2A}$ receptor binding, different benefit-of-doubt strategies have been applied, the most extreme being inclusion of men only (Bhagwagar et al., 2007; Lundberg et al., 2007; Takano et al., 2007). Hormonal therapy, particularly hormonal contraception, is common at least in western cultures, e.g. in the Danish population 44% of women, aged 15–45 years, use hormonal contraception, and 7% of women, >40 years, use hormonal replacement therapy (data from the Register of Medicinal Product Statistics at the Danish Medicines Agency and Statistics, www.medstat.dk). Therefore, restricting studies to include only women off hormonal therapy would bias the studies heavily. Hence, it is important to explore whether, especially hormonal contraception, influences cortical 5-$HT_{2A}$ receptor binding.

Here, we investigate the effects of gender and hormonal contraception in premenopausal women on cortical 5-$HT_{2A}$ receptor binding, as measured with 18F-altanserin PET.

## EXPERIMENTAL PROCEDURES

One hundred thirty-two healthy volunteers, 87 men and 45 women, with a mean age of 40.6±18.7 years (range 18–79 years), underwent 5-$HT_{2A}$ receptor imaging using 18F-altanserin-PET. None of the subjects had present or prior neurological or psychiatric disorders, nor did they have first degree relatives with any such disorder. Drug use was evaluated by interview prior to inclusion. All participants were lifetime naive for antipsychotics and antidepressants. None of the subjects had used psychostimulants other than alcohol or tobacco within the last month prior to inclusion. None of the subjects had present or prior drug-abuse. Women on hormonal replacement therapy were not included. Fourteen premenopausal women used hormonal contraception which consisted of 20 or 30 $\mu$g of ethinylestradiol combined with a progestogen (0.10–0.75 mg gestodene, 3 mg drospirenone, or 0.15 mg desogestrel). Three participants used non-sedative antihistamines known not to affect 5-$HT_{2A}$ receptor binding (O'Connor and Roth, 2005). The study was approved by the local Ethics Committee, Copenhagen, Denmark. Written informed consent was obtained from all participants. Receptor binding data (Adams et al., 2004) and positive correlations between 5-$HT_{2A}$ receptor binding and neuroticism in subsets of this group have been reported previously (Frokjaer et al., 2008).

Imaging was performed according to Pinborg et al. (2003). Subjects received a maximum dose of 3.7 MBq/kg body weight [$^{18}$F]altanserin as a combined bolus-infusion. Subsequently, they were scanned in steady state conditions for 40 min. Acquisition procedures, reconstruction, including attenuation correction, and scatter correction, and quantification are described in detail elsewhere (Pinborg et al., 2003). Structural brain imaging with magnetic resonance (MR) was conducted in all subjects; MPRAGE sequences were acquired on either a 1.5 T Vision scanner ($n$=69) or a 3 T Trio scanner (both Siemens, Erlangen, Germany) ($n$=63). PET and MR images were co-registered by the same person, and volumes of interest were delineated in a strictly user-independent fashion as described by Svarer et al. (2005). Since previous findings point at global effects on cortical 5-$HT_{2A}$ receptor binding of hormonal replacement in menopause (Moses-Kolko et al., 2003) a region representing mean cortical binding, and mean frontal cortical binding, and a region of comparison, the occipital cortex, were chosen as volumes of interest. Post hoc analyses were performed on a subdivided region set as defined in (Svarer et al., 2005) to test for lateralization and more localized effects.

The outcome parameter of specific receptor binding, $BP_P$, is defined as