Chapter 1

General Introduction
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Menopause represents a complete cessation of ovarian function leading to a sharp decrease in the circulating estrogens at the end of the reproductive life in females. The most common signs of menopause are hot flashes, headaches and mood swings, for which about 35% of the women seek medical treatment. After menopause, women have an increased risk of developing osteoporosis, heart disease and urinary tract infections. Menopause is also associated with psychological changes in women, including an increased risk of anxiety, irritability, stress, memory loss, lack of concentration and loss of libido. Eventually, this may culminate into depression. It has been reported that estrogen replacement therapy in post-menopausal women reduces anxiety and the risk of depression, is beneficial for cognition and provides neuroprotection. Estrogens replacement supplemented with androgen treatment can induce greater improvement in psychological and sexual symptoms in postmenopausal women.

Analogous to the decline in estrogen levels in menopausal women, the levels of circulating androgens decrease in middle-aged man, resulting in a hypogonadism called the “andropause”. Andropause may lead to a decrease in cognitive functions, which was suggested to be associated with depression and Alzheimer’s disease. However, the exact role of reduced androgen levels in these disorders is not well known. Treatment of hypogonadal men with the androgen testosterone was found to elevate mood and cognitive abilities.

Thus, in both men and women a reduction in the circulating sex steroid hormone (androgens or estrogens) is associated with psychological changes and replacements of these hormones are known to alleviate these psychological symptoms. Both androgens and estrogens bring about their physiological effects through their respective sex steroid hormone receptors in the brain. A reduction in the circulating sex hormones might affect the expression of their receptors. However, very little is known about the changes in the expression of the sex steroid receptor in the living brain due to changes in the levels of circulating sex steroid hormones. To better understand the role of androgen and estrogen receptors in the brain, it is necessary to in vivo quantify the expression of these receptors in health and disease.

Estrogens

Estrogens are female sex hormones that include estrone, estradiol and estriol produced in the ovaries, placenta, adrenal cortex and testes in males. Estrogens are primarily responsible for sexual differentiation, development and maintenance of secondary sex characteristics and reproduction in females. In addition, estrogens play a vital role in several other biological functions in both males and females. In the central nervous system (CNS), estrogens were found to affect numerous brain functions, ranging from fine motor control to pain sensitivity and memory. Estrogens are suggested to protect against several neurological and psychiatric disorders, including Alzheimer’s disease, Parkinson’s disease, multiple sclerosis and depression. Estrogens are used as contraceptives or for treatment of bone loss and post-menopausal symptoms (hot flashes, vaginitis and osteoporosis).
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**Estrogen receptors**

Estrogen receptors belong to the nuclear receptor superfamily. Estrogen receptors can be divided into intracellular nuclear estrogen receptors (ER) and heptahelical transmembrane G protein-coupled estrogen receptors\(^{19,20}\). The nuclear ER produce their biological effects through a slow genomic pathway, whereas the immediate effects of estrogens are thought to be processed by the membrane-bound receptors\(^{19,20}\). ER can be further subdivided into two subtypes: estrogen receptor alpha (ERα, 66kDa) and estrogen receptor beta\(^{21}\) (ERβ, 59 kDa). The ER subtypes vary in their protein length; 595 amino acids for ERα and 530 for ERβ. In addition to these two ER subtypes, several splice variants have been reported in human, which include the 2 most referred ERα splice variants, ERα46 and ERα36, and 5 ERβ splice variants\(^{22}\) (ERβ1, ERβ2, ERβ3, ERβ4, and ERβ5). Both ER subtypes have a widespread distribution throughout the body (Figure 1A). Under physiological conditions, the ER subtypes can elicit different biological responses; sometimes they can even induce opposite actions\(^{23}\). The expression of the ER subtypes can vary from organ to organ; some organs express both the ER subtypes in equal concentration, but in other organs one subtype predominates over the other\(^{24}\). In the classical ER expressing tissues, such as uterus, mammary gland, bone and cardiovascular systems, ERα predominates, whereas ERβ predominates in non-classical ER-expressing tissues, such as the prostate. Both ER subtypes have a widespread, largely non-overlapping distribution in the brain\(^{23,25}\). In rodents, the expression of both ER subtypes are mainly observed in the rostral-caudal region of the brain, where the receptors play an important role in sexual and reproductive behavior, cognition, memory and depression\(^{26–28}\) (Figure 2).

![Figure 1](image-url)  
*Figure 1:* Distribution of estrogen receptors (A) and androgen receptors (B) in the human body (Adapted from Nilsson S, Gustafsson JÅ, 2011).
In human brain, both ER subtypes are expressed predominantly in limbic system. ERα predominates in the hypothalamus and amygdala where it is involved in autonomic and reproductive neuroendocrine functions as well as emotional interpretation and processing, while ERβ predominates in the hippocampus, entorhinal cortex and thalamus, where it is involved in cognition, non-emotional memory and motor functions.

**Estrogens and depression**

Women are more at risk for reduced cognitive function, anxiety and depression than men. A reduction in the circulating estrogens after menopause has been associated with reduced mental functions (see chapter 2, 5 and 6). Abrupt changes in the mood of women have been frequently reported in the premenstrual syndrome, post-partum and during the transition between pre-menopausal to post-menopausal state. These apparent changes in the mood of women are correlated to changes in the circulating estrogens. These hormonal changes are also thought to be responsible for changes in lifestyle, sexuality and glucose metabolism. In addition psychological distress or stress in general (physical or emotional) may aggravate the depressive symptoms in post-menopausal women with reduced circulating estrogens.

Estrogen replacement can alleviate the signs of depression in peri-menopausal women to a similar extent as the standard antidepressant drugs. When estrogen replacement is initiated too late (i.e. after the post-menopausal state is already established), however, the treatment cannot establish a similar antidepressant effect anymore. These observations give the impression that there is a “critical time window” during menopausal transition, in which estrogen replacement has to be initiated in order to achieve beneficial antidepressant effects. Moreover, several other
factors need to be taken into consideration for a customized estrogen replacement regime, including the age of the subject, the time since menopause and risk factors such as the risk for stroke, breast cancer and coronary heart diseases.

From the literature it is evident that dysfunction of monoaminergic neurotransmission plays an important role in mediating the pathophysiology of depression. These neurotransmitter systems (serotonin and dopamine) are localized in the limbic brain regions involved in the regulation of mood, cognition, attention, motor activity, and fine motor skills. Estrogens interact with these neurotransmitter systems, which may explain the antidepressant effects of estrogens.

Estrogen treatment has been found to increase the concentration of serotonin in brain areas such as dorsal raphe nucleus, and hippocampus in ovariectomized rats and in hypothalamus in guinea pigs. Estrogen replacement therapy in post-menopausal women has been found to raise the levels of plasma serotonin. Estrogens may raise the levels of serotonin pre- and post-synaptically by several mechanisms. Firstly, estrogens can regulate the enzymes tryptophan hydroxylase 1 and 2, which are rate limiting enzymes for the synthesis of serotonin. Secondly, estrogens can increase the serotonin/5-hydroxyindole acetic acid ratio by inhibiting monoamine oxidase (MAO), which is an enzyme responsible for the breakdown of monoamines especially in amygdala and dorsal raphe. Thirdly, estrogen can down-regulate the expression of serotonin transporters, specifically in the basolateral amygdala, frontal cortex and some thalamic nuclei. Thus, estrogens can affect the synthesis, release and degradation of serotonin, leading to an overall net increase in the postsynaptic serotonin concentration. In addition, estrogen treatment has been found to affect the serotonin receptor densities, in particular 5HT1A, 5HT1B, 5HT2A/2C and 5HT3 in the estrogen receptor rich regions of the brain, such as hypothalamus, preoptic area and amygdala. Acute estrogen treatment increases 5HT2A receptor density and decreases the 5HT1A receptor concentration in the areas of the brain highly innervated with serotonergic projections. In contrast, chronic estrogen treatment decreased the density of 5HT2A receptors in post-menopausal women. There is evidence suggest that the antidepressant effect of the estrogens can be mediated by the desensitization of the 5HT1A receptors.

There is also evidence that the antidepressant effects of estrogens can be mediated by affecting the levels of dopamine in the synaptic cleft. There are some studies which show that treatment with estrogens can regulate the dopaminergic neurotransmission by affecting the biosynthesis, re-uptake and degradation of dopamine in areas of the brain that are targets for many antidepressant drugs like hippocampus and the frontal cortex. Estrogens can increase the post-synaptic concentration of noradrenalin by regulating the expression of i) tyrosine hydroxylase, an enzyme that catalyzes the production of noradrenalin from dopamine, ii) MAO, which is an enzyme responsible for the breakdown of monoamines, and iii) noradrenergic transporters. Moreover, estrogen treatment in the ovariectomized rats was found decrease the expression of alpha-2 adrenergic receptors, which may be the result of inhibition of noradrenalin re-uptake, as a similar effect was observed with
the serotonin re-uptake inhibitor desipramine. Additionally, estrogens can affect the expression of both dopamine D₁ and D₂ receptors and dopamine transporters (DAT). A down-regulation in the dopamine D₁ and D₂ receptors density and an up-regulation of DAT expression was observed in female rats after ovariectomy. The effect of ovariectomy could be reversed by treatment with estrogens. In summary, estrogens can affect several monoaminergic pathways by increasing the synaptic concentrations of the monoamines. Changes in post-synaptic monoamine concentrations can lead to changes in mood and behavior.

Figure 3: Effect of estrogens on different neurotransmitter systems (Adapted from McEwen BS: Estrogen Effects on the Brain: Much More than Sex. Karger Gazette Hormones 2005;66:1-9).

Androgens
Androgens are a group of male sex hormones that include testosterone, dihydrotestosterone (DHT) and androstenedione. Androgens are mainly produced in the Leydig cells of the testes. Testosterone is produced from androstenedione by a reaction that is catalyzed by 17-beta-hydroxysteroid dehydrogenase. The synthesis of testosterone not only take place in the testes, but also within the brain. Within the human brain, 17-beta-hydroxysteroid dehydrogenase is expressed in the temporal lobe and hippocampus. DHT is the active metabolite of testosterone and is 2.4 times more potent than testosterone. DHT is produced by reduction of testosterone via a reaction that is catalyzed by the enzyme 5-alpha-reductase. Androgens have both androgenic and anabolic effects. Androgenic effects include fetal and pubertal
sexual development in males, whereas anabolic effects include increase in lean body mass. Androgens are also associated with many other biological functions, such as the development and maintenance of CNS functions. The most predominant function of androgens on the brain involves the regulation of libido and aggression.

**Androgen receptors**

Androgen receptors (AR) share similar structural features with other nuclear hormone receptors. AR exist in two isoforms, AR-A (87kDa) and AR-B (110kDa), that are activated by binding of testosterone or dihydrotestosterone. Most of the effects produced by the AR are by the classical genomic pathway. Upon activation by an androgenic hormone, AR form dimers, enter the nucleus and bind to specific regions in the promoter of androgen responsive genes, resulting in the moderation of the transcription of these genes. However, some rapid effects of androgens are known to be mediated by non-genomic pathways. Limited information is available about the structural features of the membrane-bound androgen receptors.

AR have a widespread distribution in the body and brain (Figure 1B) and exert effects on multiple targets. Information on the expression of AR in the human brain is sparse. Most research on the expression of AR in the brain has been performed on rodents and non-human primates. In rats, hamster, monkeys, and baboons, AR are mainly expressed in the amygdala, hippocampus, bed nucleus of stria terminalis, preoptic area, hypothalamus, and cortical areas. Few reports on AR expression in the post-mortem human brain and in biopsy samples from the epileptic patient undergoing surgery are available. These studies indicate that AR is mainly expressed in hypothalamus, hippocampus and temporal cortex.

**Androgens and depression**

With increasing age, a progressive decrease in the concentration of circulating androgens is observed and this decrease in hypogonadal function leads to signs of depression. Testosterone treatment was found to improve mood and cognitive abilities in hypogonadal men. In post-menopausal women, combined treatment with androgen and estrogens caused a greater improvement in psychological (lack of concentration, depression, and fatigue) and sexual symptoms (decreased libido and inability to have an orgasm). Androgens may produce these beneficial effects by modulating the hypothalamic-pituitary-adrenal (HPA) axis and can also directly interact with various monoaminergic neurotransmitter systems like the serotonergic, dopaminergic and noradrenergic system. Androgens can produce their antidepressant effect through interference with serotonergic neurotransmission by an antagonistic interaction with the 5HT3 receptors, which have been suspected to play a major role in the pathophysiology of depression. In addition, castration of male rats not only decreased the levels of testosterone and estrogen, but also increased the density of the 5HT2A receptors in the forebrain, suggesting that these steroid hormones are involved in the regulation of the expression of the 5HT2A receptors. Androgens were found to increase the synaptic concentration of...
dopamine and noradrenalin and thus have a modulatory effect on the dopaminergic and noradrenergic neurotransmitter systems\textsuperscript{89,90}. Despite these preclinical results, the exact mechanism for the antidepressant effect of androgens in humans is still not clear.

Most of the above mentioned studies used invasive techniques to study the mechanism of action of estrogens or androgens on mood and behavior. Since the physiological effects of estrogens or androgens are mediated through their respective steroid hormone receptors, it may be helpful to quantify the expression of these steroid hormone receptors in the brain in a non-invasive manner. Positron emission tomography (PET) could be a suitable tool for studying the dynamic changes on estrogen or androgen receptor availability in various parts of the brain that are expected to occur during depletion and replacement of estrogens or androgens.

What is positron emission tomography?

PET is a non-invasive imaging technique used in nuclear medicine to quantitatively measure functional and metabolic processes in vivo. PET is based on the detection of very small quantities (nano- to picomolar) of a substance that is labeled with a positron emitter (radionuclide) within the body. This positron-emitting radionuclide is usually produced by a cyclotron and incorporated into a biologically active molecule with the desired physiological properties (e.g. drugs, neurotransmitters, hormones, enzyme substrates). Carbon-11, oxygen-15, and fluorine-18 are the most commonly used radionuclides for PET imaging in the brain. These radionuclides have relatively short half-lives: 2 min for oxygen-15, 20 min for carbon-11 and 110 min for fluorine-18. After incorporation of the radionuclide into the biologically active compound, the radiotracer can be injected into the blood stream of a living subject. The radiotracer will distribute throughout the body and participate in the biological process it was designed for. The biological processes could for example be binding to a receptor, metabolism into an active metabolite that is trapped inside the cell, transport across a membrane by a transporter, blood flow, perfusion, etc. The radionuclide that is incorporated into the radiotracer will undergo radioactive decay and emits a positron, which is the anti-particle of an electron (i.e. the positron has the same mass as the electron but an opposite charge). The positron travels a short distance (approximately 0.6 mm for fluorine-18 to 2.5 mm for oxygen-15) through the tissue. When the positron has lost most of its energy, it combines with an electron and annihilates, generating a pair of gamma photons with an energy of 511 keV each. Because of the conservation of momentum, the gamma photons travel in opposite directions (at an angle of 180 degrees). These photons can be detected by a pair of opposite detectors in the detector ring of the PET camera. The coincidence electronics of the camera only register the event when two detectors are almost simultaneously hit by a photon. The line between these detectors is called the line of response. Combination of multiple lines of response can provide the location of the radioactivity within the subject under investigation\textsuperscript{91}. The data acquired from the PET camera are then reconstructed to generate a 3D image of the radioactivity distribution within the body over time. The generated 3D images can be applied to
assess the radiotracer distribution within the body or the kinetics of the radiotracer at a specific location over time. Figure 4 represents a schematic illustration of the principle of PET imaging.

The most versatile and frequently used PET tracer worldwide is 2-[\(^{18}\text{F}\)]fluoro-2-deoxyglucose ([\(^{18}\text{F}\)]FDG). [\(^{18}\text{F}\)]FDG is used for measuring glucose metabolism in several medical disciplines for diagnosis, staging and therapy monitoring of patients with e.g. cancer, infections, cardiac infarction and neurological disorders, like Parkinson’s disease and dementia^{92–94}. Measurement of glucose utilization during a depressive state may also be a suitable biomarker for the severity of depression.

**Aim and outline of the thesis**

The estrogens and androgens are thought to play an important role in depression. Estrogens and androgens produce their physiological effects through activation of ER and AR, respectively. To better understand the mechanism of action of these sex steroid hormones on brain function, it is helpful to know the expression of their receptors in the living brain.

![Figure 4: Basic principle of Positron Emission Tomography (Copied from Jens Maus master thesis (Wikipedia)).](image)

Measurement of the expression of ER and AR can be performed by PET using the radiotracers 16α-[\(^{18}\text{F}\)]fluoro-17β-estradiol ([\(^{18}\text{F}\)]FES) or 16β-[\(^{18}\text{F}\)]fluoro-5α-
dihydrotestosterone ([\(^{18}\text{F}\)]FDHT), respectively. Besides \([^{18}\text{F}\)]FES and \([^{18}\text{F}\)]FDHT, several other tracers have been developed for imaging of ER and AR, but these tracers have not been widely applied yet. So far, PET imaging of ER and AR has only be performed in patients with hormone sensitive cancer, like breast cancer and prostate cancer, respectively. So far, none of the tracers for ER and AR have been applied for assessing steroid hormone receptor expression and availability in the brain. The aim of the first part of this thesis was therefore to assess whether the existing PET tracers for steroid hormone receptors could be applied for imaging of steroid hormone receptors in the rat brain. In the second part of this thesis, the role of estrogen in depression was studied in ovariectomized rats.

This thesis starts with a review on steroid hormones and their receptors in the healthy and diseased brain (Chapter 2). This chapter also contains an overview of the available PET tracers for imaging of steroid hormones receptors and the potential utility in brain steroid hormone receptor imaging.

Studying the expression of AR in the brain by PET imaging, could provide a better understanding of the physiological effects of androgens. \([^{18}\text{F}\)]FDHT is a tracer that was developed to image AR in prostate cancer. It may also be potentially suitable for imaging AR in the brain, but this has not been investigated yet. In Chapter 3, the utility of \([^{18}\text{F}\)]FDHT for AR imaging in the brain was therefore evaluated in orchiectomized rats that are deficient in endogenous androgens.

Estrogens are known to play a role in depression, neuroprotection and cognition. \([^{18}\text{F}\)]FES is a PET tracer that is used in clinical practice to image ER in breast cancer, but its potential suitability for imaging of ER in the brain has not yet been investigated. In Chapter 4, it was investigated if \([^{18}\text{F}\)]FES could be used for imaging of ER in the rat brain. For this purpose, we used female rats at different stages of the estrous cycle, ovariectomized female rats and male rats, in order to also investigate the influence circulating estrogens levels on \([^{18}\text{F}\)]FES uptake in the brain.

It has been shown that estrogen depletion can induce depression. In Chapter 5 we assessed the effect of an ovariectomy-induced reduction in circulating estrogen levels on depressive-like behavior, as measured by the forced swim test, and on brain glucose metabolism, as determined by \([^{18}\text{F}\)]FDG PET. Additionally, we investigate the anti-depressant effect of immediate and delayed estradiol replacement, using treatment with anti-depressant drugs as a positive control.

We anticipated that the effect of estrogen depletion on depression could be reinforced by stress. Chronic mild stress (CMS) is a widely investigated model for depression that replicates symptoms of human depression and anhedonia. In Chapter 6, we assessed the effect of ovariectomy and estradiol replacement on depressive-like behavior in rats before and after exposure to CMS. Depressive-like behavior was assessed using the sucrose consumption test, the open field test and the forced swim test. Brain glucose metabolism was assessed by \([^{18}\text{F}\)]FDG PET.

Finally, the findings from this thesis are summarized in Chapter 7. Chapter 8 provides some future perspectives based on the results of this thesis.
References

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