

From the Department of Women's and Children's Health,  
Division of Obstetrics and Gynecology,  
Karolinska Institutet, Stockholm, Sweden

**TESTOSTERONE AND ESTROGEN TREATMENT  
IN POSTMENOPAUSAL WOMEN –  
ASPECTS ON BEHAVIOR AND BRAIN FUNCTION**

**Ljiljana Kočoska-Maraš**



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*To my family*



*„Јас го разбираам светот единствено како поле за културен натпревар меѓу народите”*  
-Гоце Делчев

*“I understand the world solely as a field for cultural contest among the nations”*  
-Goce Delčev

# ABSTRACT

The human brain can be regarded as a target organ for sex steroid hormones. Hormones exert their influence via different pathways and sex steroid receptors are widely distributed within the brain. Several studies suggest gender differences in behavior and cognitive function and have stimulated research on the role of testosterone and estrogen. The overall aims of this thesis were to explore eventual effects of sex hormone treatment on certain aspects of behavior and brain function in postmenopausal women.

A total of two hundred naturally postmenopausal women were recruited to four weeks of treatment with either testosterone undecanoate 40 mg/day, estradiol valerate 2 mg/day or placebo in a randomized trial. At the end of treatment, all women were tested to evaluate economic behavior (altruism, reciprocal fairness, trust, trustworthiness, risk aversion, risk investment, risk assessment) and cognitive function (verbal memory, verbal fluency, spatial ability). Blood samples were collected at baseline and after four weeks and analyzed for estradiol, testosterone, androstenediol glucuronide, oxytocin, sex hormone-binding globulin and insulin-like growth factor I.

Treatment with testosterone or estrogen had no significant influence on economic behavior and cognitive function. Still, significant correlations between sex hormone levels and some aspects of cognitive function were found. High estrogen levels, a high estradiol/testosterone ratio and increasing estradiol levels during estrogen treatment were all associated with lower spatial ability. It could be that a specific balance between estrogen and testosterone is required for optimal effects on spatial- and verbal abilities. Also the estrogen/testosterone ratio and a curvilinear relationship could be important.

Ten surgically postmenopausal women were treated with estrogen alone (transdermal estradiol 100µg/day) for three months and in combination with testosterone undecanoate 40 mg/day for further three months. The influence of treatment on the serotonin transporter binding potential (5-HTT BP) in specific brain areas was studied by positron emission tomography (PET) using the special ligand [<sup>11</sup>C]MADAM. Serum levels of sex hormones, mood and cognitive abilities were measured.

Treatment with estrogen alone or in combination with testosterone significantly reduced 5-HTT BPs in several cortical and limbic regions. Furthermore, hormone treatment significantly enhanced mood and cognitive abilities like letter and category fluency. These data provide novel evidence for the influence of sex steroid hormones on the serotonergic system in the human brain.

**Key words:** cognitive function, economic behavior, estrogen, positron emission tomography, postmenopausal women, serotonin transporter, testosterone

## LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals *I-IV*:

- I. Zethraeus N, Kočoska-Maraš L, Ellingsen T, von Schoultz B, Hirschberg AL, Johannesson M. A randomized trial of the effect of estrogen and testosterone on economic behavior. *Proc Natl Acad Sci* 2009; 21;106:6535-8.
- II. Kočoska-Maraš L, Zethraeus N, Rådestad AF, Ellingsen T, von Schoultz B, Johannesson M, Hirschberg AL. A randomized trial of the effect of testosterone and estrogen on verbal fluency, verbal memory, and spatial ability in healthy postmenopausal women. *Fertil Steril* 2011; 95:152-7.
- III. Kočoska-Maraš L, Rådestad AF, Carlström K, Bäckström T, von Schoultz B, Hirschberg AL. Cognitive function in association with sex hormones in postmenopausal women. *Gynecol Endocrinol* 2012 Sep 11 [Epub ahead of print].
- IV. Kočoska-Maraš L, Jovanović H, Rådestad AF, Hirschberg AL, Nordström A-L. Effects of estrogen and testosterone treatment on serotonin transporter binding in the brain of surgically postmenopausal women – A PET study [manuscript].

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## LIST OF ABBREVIATIONS

A $\beta$	amyloid beta
ADG	androstanediol glucuronide
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AR	androgen receptor
BAI	Beck Anxiety Inventory
BP	binding potential
BDI	Beck Depression Inventory
BMI	body mass index
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
DHT	dehydrotestosterone
E	estrogen treatment
ER	estrogen receptor
ERE	estrogen-response element
GABA	gamma-aminobutyric acid
HRT	hormone replacement therapy
PET	positron emission tomography
5-HIAA	5-hydroxyindole-3-acetic acid
5-HTT	serotonin transporter
IGF-I	insulin-like growth factor I
MADAM	N,N-dimethyl-2-(2-amino-4-methylphenylthio) benzyl amine
MINI	Mini-International Neuropsychiatric Interview
MRI	magnetic resonance imaging
PET	positron emission tomography
PMDD	premenstrual dysphoric disorder
PR	progesterone receptor
ROI	regions of interest
SHBG	sex hormone-binding globulin
SSRI	selective serotonin reuptake inhibitor
SRTM	simplified reference tissue model
T	testosterone treatment
TAC	time activity curve
TMT-A	trail making test A
TMT-B	trail making test B
VOI	volume of interest
WHIMS	Women's Health Initiative Memory Study

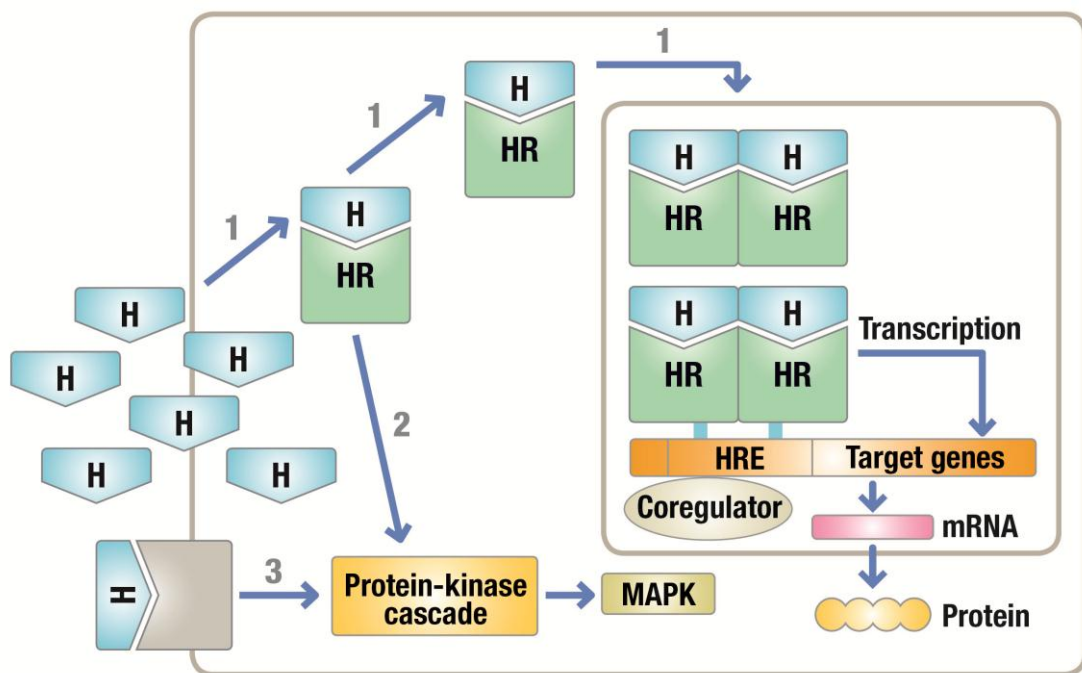


# 1 INTRODUCTION

## 1.1 THE BRAIN – A TARGET ORGAN FOR SEX STEROIDS

### 1.1.1 Sex steroid receptors in the brain

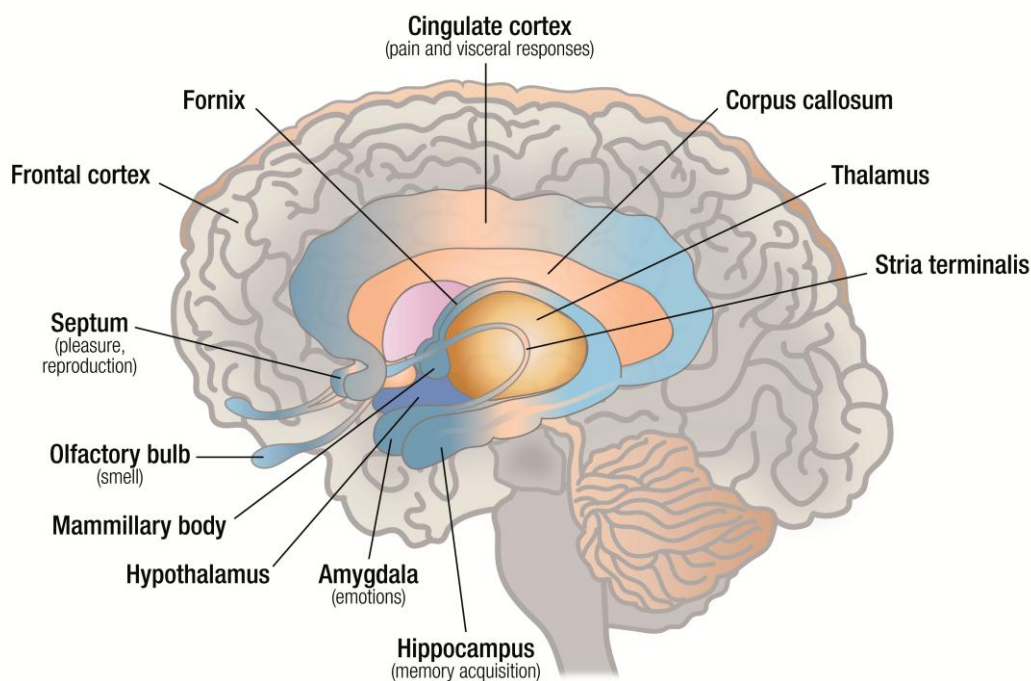
The human brain can be regarded as a target organ for sex steroid hormones. Estrogen, progesterone and testosterone display their effects via genomic mechanisms by binding to nuclear receptors and nongenomic mechanisms (Fig 1). The specific receptors for estrogens (ER $\alpha$ , ER $\beta$ ), progesterone (PRA, PRB) and androgens (AR) all belong to the same family, the nuclear receptor superfamily (Genazzani et al 2002, Birzniece et al 2006). After ligand binding there is regulation of gene expression and the production of proteins and several neurotransmitters is initiating (McEwen 2002).



**Figure 1.** Mechanisms of steroid hormone (H) action. Pathway 1 shows direct genomic actions via binding to nuclear receptors (HR). The hormone-receptor complex binds to the hormone response element (HRE) located in target genes leading to transcriptional activation and subsequently to protein synthesis. Pathway 2 demonstrates indirect genomic mechanisms involving activation of HR linked to distinct second messenger systems (e.g. mitogen-activated protein kinase (MAPK)). Pathway 3 represents non-genomic steroid hormone actions by inducing rapid signaling via membrane-bound mechanisms.

However, the ‘nuclear hormone receptors’ are not only found within the nucleus but also in the cytoplasm, and in the cell membrane (Benmansour et al 2008). Indirect genomic mechanisms involve activation of hormone receptors linked to specific second messenger systems. Sex hormones may also influence various brain functions via more rapid, within milliseconds to minutes range, signaling events i.e. nongenomic mechanisms by changing cell membrane permeability and the influx of ions and signal substances (McEwen 2001, Simoncini and Genazzani 2003).

Some brain regions exclusively express ER $\alpha$ , such as ventromedial nucleus of the hypothalamus and also the subfornical organ, which is part of the circumventricular organs in the brain (Fig 2). These areas are involved in feeding, fear, regulation of fluid and electrolyte balance, thermoregulation, and sexual activity (ter Horst 2010). In contrast, neurons in the olfactory bulb, supraoptic nucleus, paraventricular and tuberal hypothalamic nuclei, as well as cerebellum exclusively express ER $\beta$  (ter Horst 2010). Higher levels of ER $\beta$  are also found in the hippocampus, thalamus, and cerebral cortex suggesting the role of ER $\beta$  in cognition and memory (Shughrue et al 1997, Österlund and Hurd 2001).



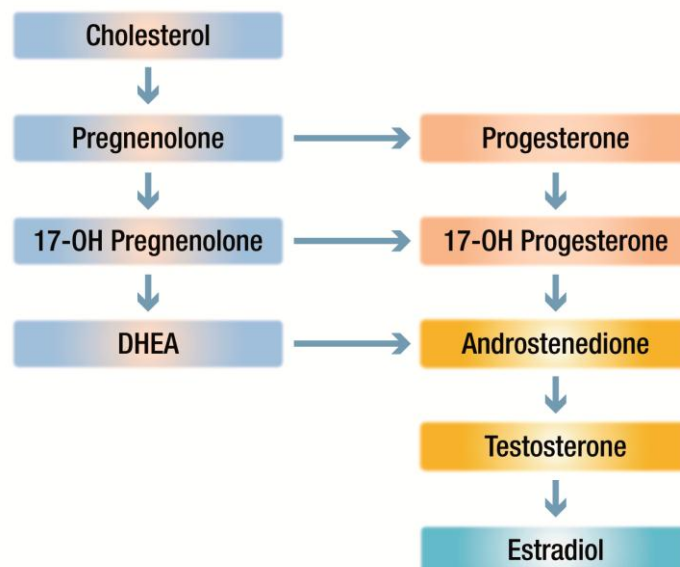
**Figure 2.** Sagittal view of different regions of the human brain. The limbic system is composed of tightly interconnected brain areas e.g. cingulate cortex, anterior thalamus, hippocampus, amygdala, mammillary bodies, septum and olfactory bulb.

Progesterone receptors are also distributed in many brain areas (Bethea and Widmann 1998, Greco et al 2001). There is often co-expression of ER $\alpha$ , ER $\beta$ , and PR immunoreactivity. Administration of estradiol will decrease ERs but increase progesterone receptor expression in the brain (Alves et al 1998, Greco et al 2001).

Likewise, ARs are widely distributed in the brain. Animal and human studies have demonstrated the presence of AR in various areas in the brain, mainly in hypothalamus and the limbic system (McEwen et al 1980), as well as in the preoptic area and substantia nigra (Bixo et al 1995, Steckelbroeck et al 1999).

### 1.1.2 Sex steroid production and action

The main source of estradiol and progesterone production is granulosa cells and corpus luteum of the ovaries during the reproductive life (ter Horst 2010). In the ovaries, conversion of cholesterol into pregnenolone occurs which is then transformed to the androgens androstenedione and testosterone, which in turn can be further converted to estradiol by the enzyme aromatase (Fig 3).



**Figure 3.** Sex steroid hormone production in the ovary.

Androgens are secreted by both the ovaries and adrenal glands in women. Each source contributes to about 50% of total testosterone production. About 50% of circulating testosterone is produced by peripheral conversion of adrenal and ovarian androgens (Crilly et al 1981), mainly from androstendione but also from dehydroepiandrosterone (DHEA) and its sulphate (DHEAS). Such conversion occurs in e.g. fat tissue, liver, muscle, and also in the brain (Birzniece et al 2006).

During the menopausal transition, cyclic secretion of estradiol and progesterone disappears and production of the female sex hormones declines rapidly. However, circulating levels of androgens decline as a consequence of age-related reductions in secretion by both the adrenal gland and the ovaries (Davison et al 2005).

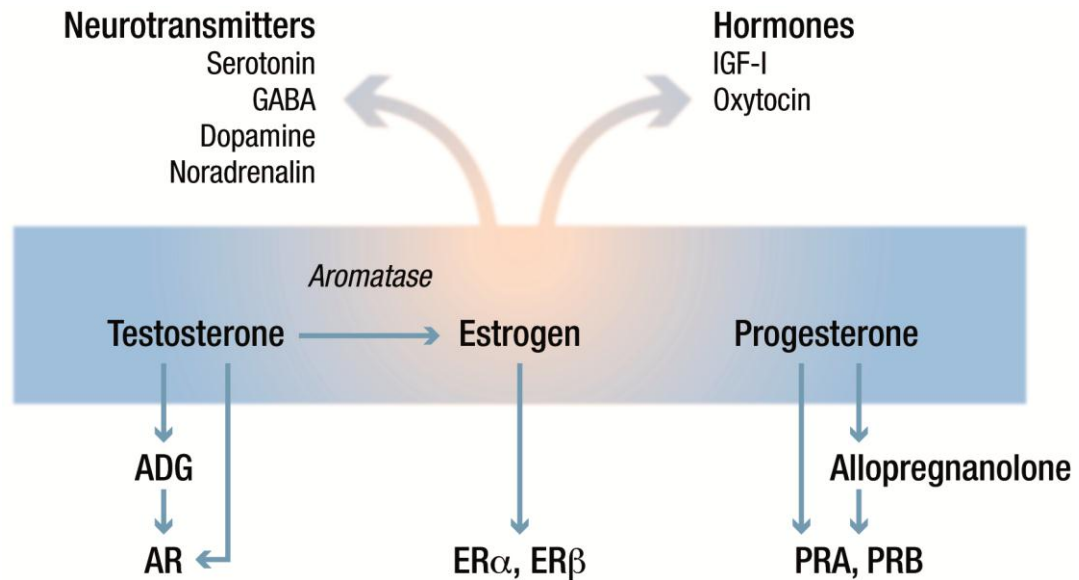
Most of circulating estradiol, progesterone and testosterone are bound to plasma proteins such as sex hormone-binding globulin (SHBG), albumin and transcortin (Speroff et al 1999). Only a few percent of the total hormone concentration is unbound and free to pass the cell membrane and exert its biological effect.

Steroid hormones are lipophilic and have a low molecular weight. They readily cross the blood–brain barrier. An accumulation of these hormones in the brain compared to circulating concentrations occurs (Birzniece et al 2006). However, the distribution in the brain is not even and certain brain regions accumulate more than others. Steroid hormone concentrations in the brain vary in association with circulating concentrations throughout the menstrual cycle (Bixo et al 1997).

Estrogen, progesterone, testosterone and their metabolites can be classified as neurosteroids since they can be synthesized in the central and peripheral nervous system (Compagnone and Mellon 2000). Enzymes needed for estradiol synthesis, P450 17 $\alpha$  and P450 aromatase, have been identified in different brain areas e.g. hippocampal neurons (Hojo et al 2004). In an animal study, the aromatase P450 inhibitor formestane was found to decrease estradiol concentrations in the cortex (Amateau et al 2004).

Sex steroids may act directly like estradiol by binding to its specific receptors (Fig 4). Testosterone also exerts direct actions via AR (Mooradian et al 1987, Zuloaga et al 2008) or can be converted to estradiol by the enzyme aromatase in both peripheral tissue and in the brain (Simpson 2002). A high aromatase activity has been reported in

several areas of the rat brain (Roselli and Resko 1993). Effects of sex steroids are furthermore mediated via active metabolites such as 5 $\alpha$ -dihydrotestosterone (DHT) and androstandiol glucuronide (ADG) for testosterone (Brand and van der Schouw 2010, Reddy and Jian 2010) and allopregnanolone for progesterone (Compagnone and Mellon 2000).



**Figure 4.** Principles of steroid hormone actions in the brain. Sex steroids may act directly by binding to specific receptors or via active metabolites. Ovarian hormones may also display their effects indirectly through neurotransmitter systems or other hormonal systems. ADG = androstandiol glucuronide, AR = androgen receptor, ER = estrogen receptor, GABA =  $\gamma$ -aminobutyric acid, IGF-I = insulin-like growth factor I, PR = progesterone receptor.

Ovarian hormones can also exert their effects indirectly through neurotransmitter systems including the serotonin and  $\gamma$ -aminobutyric acid (GABA) system in the brain (McEwen 2001). Moreover, sex steroids may modulate brain functions by altering circulating levels of insulin-like growth factor I (IGF-I) and its binding proteins (Cherrier et al 2004, Garcia-Segura et al 2010), as well as local expression of IGF-I in the brain (Åberg et al 2006). IGF-I has been shown to have a neuromodulatory role in cognitive function (Aleman and Torres-Aleman 2009).

Circulating levels of oxytocin are also increased by endogenous and exogenous estrogens (Shukovski et al 1989, Bossmar et al 1995). Animal and human studies reveal that oxytocin gene promoters contain estrogen-response elements (ERE) and are stimulated by estrogen (Mohr and Schmitz 1991, Gimpl and Fahrenholz 2001).

Oxytocin acts through its cognate receptor, the oxytocin receptor which is a 389-amino acid polypeptide. Beside its roll in lactation and parturition, oxytocin modulates various behaviors, including anxiety, social memory and recognition, stress response, sexual and aggressive behaviors, and maternal behavior (Lee et al 2009).

## 1.2 SEX STEROIDS AND COGNITION

Several studies suggest certain gender differences in cognitive function. In general, men tend to excel women in spatial ability tests, while women outperform men in episodic memory and verbal fluency (Lewin et al 2001, Kimura 2002, Kimura and Clarke 2002). Apparently, these findings have stimulated research on the role of estrogen and testosterone in cognitive performance. However, studies on the possible relationship between gender differences and levels of gonadal hormones yield a complex and also contradictory picture.

Some menstrual cycle studies suggest improvement in visuospatial skills when estrogen levels are low (Kimura 2002) and enhanced verbal fluency during high estradiol levels (Sherwin 2009). In contrast, other research found no difference in cognitive performance between phases of the menstrual cycle (Rosenberg and Park 2002). There were no differences in cognitive ability between groups of premenopausal, perimenopausal and postmenopausal women with different estrogen status (Herlitz et al 2007). Still, a large longitudinal study showed decrease in verbal memory during the menopausal transition (Greendale et al 2009). Likewise, correlation studies have found inconsistent results for the possible associations between levels of circulating testosterone and cognitive performance in women (Sherwin 2009).

The discrepancies in the literature are probably due to several factors e.g. individuals of varying age and hormonal levels, differences in hormonal assays and tests used for cognitive assessment (Thilers et al 2006). The balance between estrogen and testosterone and thus the estradiol/testosterone ratio has been suggested to be more important than the absolute hormone levels (Sherwin 2009). It could also be that the association between sex steroid hormone levels and some aspects of brain function is not dose dependent in a straight linear fashion. In fact, it has been shown that several GABA receptor agonists, including the progesterone metabolite allopregnanolone, have



a biphasic dose response curve (Srinivasan et al 1999). A low dose may induce negative reactions, such as dysphoria, anxiety and aggression whereas high doses of GABA-A agonists have anxiolytic, antiepileptic and sedative effects (Backstrom et al 2011). A curvilinear relationship and an optimal level have also been suggested for the effects of testosterone on visuospatial ability in both women and men (Moffat and Hampson 1996, Muller et al 2005).

A neuroprotective effect of estrogen has been suggested for several neurological diseases such as Alzheimer's disease. Numerous *in vitro* and *in vivo* studies in both animals and humans have demonstrated beneficial effects of estrogens on Alzheimer's neuropathology (Pike et al 2009).

Alzheimer's disease is a neurodegenerative disease with progressive loss of neural function and cognitive abilities. At the cellular level it is characterized by accumulation of amyloid  $\beta$  ( $A\beta$ ) peptide, neurofibrillary tangles that consist of hyperphosphorylated tau protein and progressive neural loss (Selkoe 2001). In experimental studies, ovariectomy in rodents has been associated with increased accumulation of  $A\beta$  peptide. Estrogen supplementation was found to reverse this increase (Petanceska et al 2000, Zheng et al 2002). Estrogen may also protect against intracellular  $A\beta$  toxicity and apoptosis, as well as reduce the level of  $A\beta$  *in vitro* (Zhang et al 2004, Nilsen et al 2006).

Women have a higher risk of developing Alzheimer's disease than men (Gao et al 1998). Also circulating levels of estrogen were found to be lower in women suffering from the disease than in healthy controls (Manly et al 2000). There are data to suggest positive effects of estrogen treatment on cognitive function in healthy postmenopausal women of younger age (Sherwin 1988, Shaywitz et al 2003, Joffe et al 2006). However, in the large prospective Women's Health Initiative Memory Study (WHIMS), rather an adverse effect was found of estrogen treatment alone (Espeland et al 2004), whereas estrogen in combination with progestin did not improve global cognitive function in postmenopausal women 65 years and older (Rapp et al 2003). The results may be explained by a protective effect of estrogen on cognition when the therapy is initiated early after menopause, but an adverse effect in older women (Sherwin and Henry 2008, Maki and Sundermann 2009). More knowledge is needed about the causal relationship between sex hormones and cognitive skills in women.

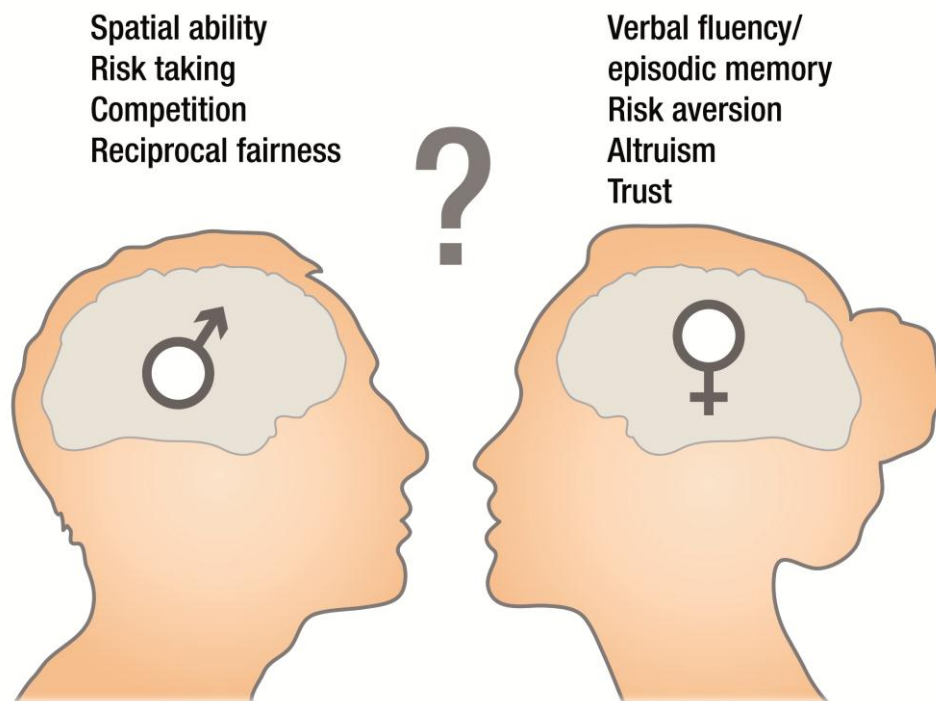
### 1.3 ECONOMIC BEHAVIOR

Behavioral economics is a novel multidisciplinary research field studying the effects of social, cognitive, and emotional factors on economic decisions of individuals and institutions and the consequences for the market, and resource allocation (Durlauf and Blume 2008). Humans display sizeable individual variation in economic behaviors. Heterogeneity is large both in the domain of personal decision making and in the domain of social interaction. Some individuals willingly take risks that others pay to avoid (Dohmen et al 2005), and in situations where some individuals are altruistic and trusting, others are selfish and distrustful (Camerer 2003).

Relatively little is known about the sources of such preference heterogeneity, but two recent findings suggest that biological factors are important. First, comparisons of the behavior of identical and fraternal twins indicate that genetics explains a sizeable part of the variation in preferences across a wide range of economic domains. Second, a controlled increase in the level of oxytocin, that is produced in the hypothalamus and released in the brain and bloodstream, have been reported to cause more trusting behavior (Kosfeld et al 2005) indicating the importance of hormonal factors. Trust is essential in friendship, love, families, organizations (Krueger et al 2012) and facilitates interpersonal relations. It permits reciprocal behaviors that lead to mutual advantages for cooperators during economic and social exchange. Variations in trust are often assigned to attitudes or personality; one person may be described as “very trusting” and another as “mistrustful” (Krueger et al 2012). Reports from converging animal and human studies reveal that oxytocin, that functions both as a hormone and neurotransmitter, broadly influences socio-emotional behaviors e.g. trust (Lee et al 2009). Because hormone levels in general are under strong genetic influences (Harris et al 1998, Bartels et al 2003), these relationships between hormone levels and behavior suggest one possible channel for the intergenerational transmission of behavior.

In human social interplay, the ability to correctly record and also to predict the behavior of another individual is of great importance for physical and social survival (Frith and Frith 1999, van Honk et al 2004). During the evolution cognitive-empathetic mechanisms to read other peoples thoughts and emotions have been developed (Hill and Frith 2003, Realo et al 2003). The face and in particular the eyes are important sites

for such unconscious and intuitive communication (Baron-Cohen 2003). On average women have a higher ability to “read the mind from the eyes” than men (Voracek and Dressler 2006, Sapienza et al 2009) (Fig 5). This gender difference has been suggested to be associated with levels of androgens (Pennebaker et al 2004, Baron-Cohen et al 2005). Fetal programming of the brain as influenced by testosterone might be a basis for differences in behavior during adult life depending on social context (Sisk and Zehr 2005). The fetal period of prenatal development (between weeks 12 and 19 of gestation) is considered critical for testosterone's effects on brain organization, whereas activational effects come into prominence in adolescence and adulthood (DeCatanzaro 1998, Sisk and Zehr 2005).



**Figure 5.** Average differences between women and men in some aspects of behavior.

Testosterone has been reported to increase behavior of competition and dominance (Archer 2006) and to reduce fear (van Honk et al 2004, Hermans et al 2006). Testosterone has also been associated with high risk behavior e.g. gambling and alcohol use (Mazur 1995, Blanco et al 2001). However, to what extent testosterone may influence economic behavior is currently controversial (Burnham 2007, Coates and Herbert 2008, Coates et al 2009, Sapienza et al 2009).

On average, women seem to be less willing to accept high risks in financial decision-making than men (Sapienza et al 2009). Experimental evidence shows that women tend to be more risk averse, less competitive, and more prosocial than men (Croson and Gneezy 2009) (Fig 5). Two studies reveal that risk-taking behavior varies over the menstrual cycle, i.e. women are more risk averse during the ovulatory phase, when the estradiol level is high, in comparison with other cycle phases (Chavanne and Gallup Jr 1998, Bröder and Hohmann 2003).

Since men and women have sharply different levels of sex hormones, it is natural to think that hormones are implied in the differences between male and female behavior.

To investigate the causal link between sex hormones and economic behavior there is a need for placebo-controlled and double-blind experiments.

#### 1.4 SEX STEROIDS AND MOOD

Women are more likely than men to suffer from depression and anxiety disorders (Kessler 2003). The lifetime prevalence of major depression is approximately 8% in women and 4% in men. In women, depressive episodes may be longer in length, more recurrent and also associated with more functional impairment than in men (Burt and Stein 2002, Yonkers 2003). Also anxiety disorders are twice as common in women as in men (Pigott 1999). Sex differences in prevalence rates appear to be independent of country and culture and cannot be completely explained by social support, psychosocial factors or coping style (Gater et al 1998).

The reasons for these apparent gender differences have not been clarified. There is epidemiological evidence that the occurrence of depression is similar in young girls and boys and that it is changed to the 2:1 female to male ratio first after puberty and after the influence of gonadal hormones (Kessler and Walters 1998). Women report more depressive and dysphoric symptoms during times of large hormonal changes, including the premenstrual period, postpartum and perimenopause (Soares and Zitek 2008). Premenstrual dysphoric disorder (PMDD) is considered a specific psychoneuroendocrine disorder in women of reproductive age, characterized by severe cyclic mood changes during the luteal phase (Sundström et al 1999). These lines of evidence suggest that gonadal hormones might be of particular importance in women

contributing to the higher prevalence of depression and anxiety disorders compared to men. Furthermore, significant differences in brain structure and function between women and men have been demonstrated, suggesting a higher biological susceptibility to depression in females (Cahill 2006, Cosgrove et al 2007).

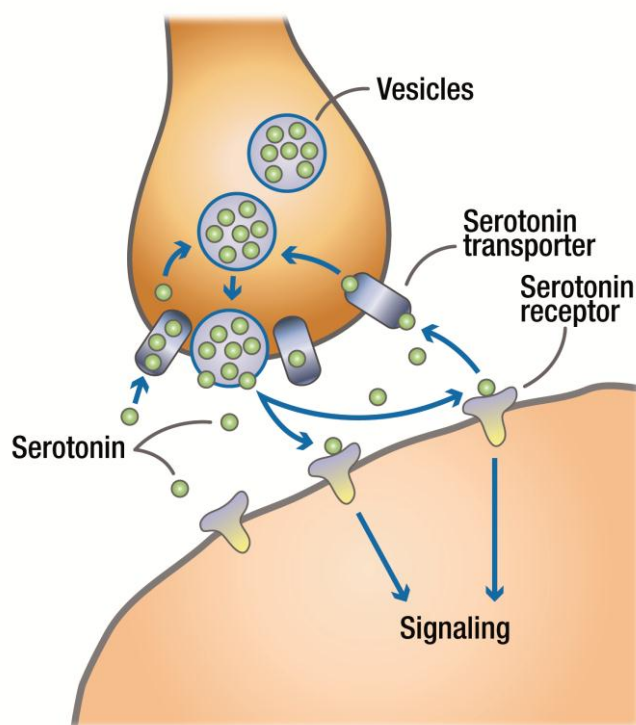
Some studies have reported a positive effect on depression by hormone replacement therapy (HRT) in perimenopausal and postmenopausal women menopausal (Zweifel and O'Brien 1997, Soares et al 2001, Frey et al 2008) but these results are contradicted by other researchers (Morrison et al 2004). Combined estrogen and selective serotonin reuptake inhibitor (SSRI) treatment has also been suggested to improve postmenopausal depression (Schneider et al 2001, Rasgon et al 2007), but again, other studies have not confirmed these results (Huttner and Shepherd 2003). Addition of testosterone to estrogen replacement therapy may also improve psychological general wellbeing as compared to estrogen treatment alone (Shifren et al 2000, Nathorst-Böös et al 2006).

## 1.5 THE SEROTONIN SYSTEM

Within the brain there are important interactions between sex steroid hormones and the central serotonergic system. Both systems are linked and involved in the regulation of mood and behavioural functions such as affect, learning, memory, sexual behaviour, aggression, stress responses, sleeping, thermoregulation and eating (Rubinow et al 1998, Birzniece et al 2006).

Serotonin-producing neurons in the raphe nuclei are targets of ovarian steroids (Sheng et al 2004) and act to facilitate serotonin neurotransmission (Bethea et al 2002). Synaptic concentrations of serotonin are regulated by the serotonin transporter protein (5-HTT) (Michopoulos et al 2011). Ovarian steroid hormones have been suggested to modulate the function of the serotonin neural system by inhibiting serotonin re-uptake through allosteric binding of the 5-HTT via nongenomic pathways (Chang and Chang 1999). Serotonin transport is implied in a variety of behavioral and physiological responses and dysfunction of serotonin neurotransmission is associated with depression, anxiety and also suicidal behavior (Arango et al 2002).

Blocking or reducing serotonin re-uptake at the synapse has been reported to alleviate depression (Smith et al 2011). The 5-HTT is a primary target for the action of SSRIs (Parsey et al 2006). SSRIs are effective treatment for major depressive disorder, posttraumatic stress disorder, generalized anxiety disorder, obsessive compulsive disorder and other mood and anxiety disorders (Shelton 2004). SSRIs bind to the transporter, block the reuptake of serotonin, and increase the synaptic serotonin concentration (Blier and de Montigny 1999) (Fig 6). Estrogen treatment may have important permissive effects on the actions of serotonergic antidepressants (Schneider et al 2001, Rasgon et al 2007).



**Figure 6.** The serotonin synapse.

The higher prevalence rates of depression and anxiety disorders in women compared to men can be explained by sexual dimorphisms in the serotonergic system (Jovanovic et al 2008). In human studies, a greater responsiveness to serotonergic challenges has been reported in women (McBride et al 1990). Furthermore, women may respond better to SSRIs than men (Kornstein et al 2000, Young 2001).

Animal studies analyzing the action of estrogen *in vivo* on 5-HTT uptake sites and mRNA levels are not conclusive. Increases, decreases, and no change in 5-HTT uptake sites have been shown (Mendelson et al 1993, Attali et al 1997, Pecins-Thompson et al 1998, McQueen et al 1999). Variability in the treatment paradigm used e.g. the time elapsed between ovariectomy and estrogen treatment, the duration of estrogen treatment, and the brain region evaluated may explain discrepancies in these studies (Benmansour et al 2009).

Until fifteen years ago, the principal methods for studying serotonin metabolism in the human brain were determination of the metabolite of serotonin 5-hydroxyindole-3-acetic acid (5-HIAA) in cerebrospinal fluid and postmortem measurements of brain serotonin and 5-HIAA (Nishizawa et al 1997). However, both methods have limitations. None of these provide a direct measure of serotonin synthesis in the living brain.

The development of positron emission tomography (PET) and of selective radioligands has made it feasible to perform *in vivo* studies of biomarkers in the human brain. This technique allows e.g. direct measurement of serotonin synthesis in various brain regions, possibility for repeated measurements of serotonin synthesis in the same subject after a short time interval (Diksic et al 1991). Furthermore, PET is less invasive than a lumbar puncture, the results are not influenced by a variety of factors unrelated to the rate of serotonin synthesis that can change cerebrospinal fluid values (Nishizawa et al 1997).

PET studies of depressed patients have supported a pathophysiological role of serotonin (Savitz et al 2009). In patients with major depression such *in vivo* imaging studies indicate reduced levels of 5-HTT binding of serotonin in the midbrain (Malison et al 1998, Parsey et al 2006) and amygdala (Parsey et al 2006). Other PET studies have also revealed the relationship between 5-HTT occupancy and blood levels of SSRIs (Meyer et al 2004).

So far, there are only a few PET studies on the effects of gonadal hormones on the human brain (Moses et al 2000, Kugaya et al 2003, Jovanovic et al 2006, Frokjaer et al 2010, Moser et al 2010). Two studies on small samples of postmenopausal women (Moses et al 2000, Kugaya et al 2003) have been performed, where PET and the

radioligand [18F] altanserin were used to study the effect of HRT on serotonin 2A receptors. An increase of serotonin 2A receptors was found in cortical brain regions by HRT. However, there are no previous studies investigating the effects of estrogen and testosterone treatment on the 5-HTT in the human brain.



## 2 AIMS OF THE THESIS

The overall aims of this Thesis were to explore eventual effects of testosterone and estrogen treatment on certain aspects of behavior and brain function in postmenopausal women.

### **Specific aims:**

- To investigate the effects of short-term low dose treatment with testosterone or estrogen in a randomized placebo-controlled trial on economic behavior in postmenopausal women.
- To evaluate the potential influence of such treatment on cognitive functions like verbal fluency, verbal memory and spatial ability in the same group of postmenopausal women.
- To analyze possible associations between serum levels of sex steroid hormones, their metabolites and verbal fluency, verbal memory and spatial ability.
- To explore the effects of estrogen treatment alone or in combination with testosterone on serotonin transporter binding potentials (5-HTT BPs) in specific brain areas of surgically postmenopausal women.

## 3 MATERIALS AND METHODS

### 3.1 SUBJECTS

In paper *I*, *II* and *III*, healthy naturally postmenopausal women in the 50–65 years age-group were recruited for study mainly based on advertisements in newspapers. An initial screening was performed by phone. Subjects that were eligible for the study participated in a comprehensive screening visit at the Women's Health Research Unit at the Karolinska University Hospital, Stockholm, Sweden. At screening, a medical and gynecological examination was performed. Two hundred and forty women were screened.

Inclusion criteria were women 50–65 years of age with body mass index (BMI) 19–30 kg/m<sup>2</sup>. Postmenopausal status was defined as last menstrual bleeding at least 12 months ago or serum levels of follicle-stimulating hormone (FSH) > 30 IU/L. Exclusion criteria were smoking, hypertension, hyperlipidemia, or other cardiovascular disease. Furthermore, risk factors for thromboembolism, diabetes, and history of cancer. Intake of sex steroid hormones during the past three months was not allowed. However, well-controlled thyroid hormone substitution for treatment of hypothyroidism was permitted.

Two hundred and three women fulfilled the criteria and participated in the study. Of those 200 women completed the study. Two women discontinued due to mild estrogen-related side effects and one woman in the placebo group discontinued due to skin reactions.

In paper *IV*, healthy surgically postmenopausal women aged 40–65 years were recruited by advertisement in a local newspaper or by letters to potential participants identified from hospital records. Inclusion criteria were hysterectomy and bilateral oophorectomy due to benign indication; at least two months between operation and inclusion; age 40–65 years and BMI < 30 kg/m<sup>2</sup>. Exclusion criteria were the same as in studies *I–III* and furthermore, psychiatric disorder, alcohol and drug abuse. HRT during the last three months was not allowed. However, well controlled thyroid hormone substitution was permitted. Fourteen women came to a screening visit at the Karolinska University Hospital for medical and gynecological examination. Also a psychiatric Mini-

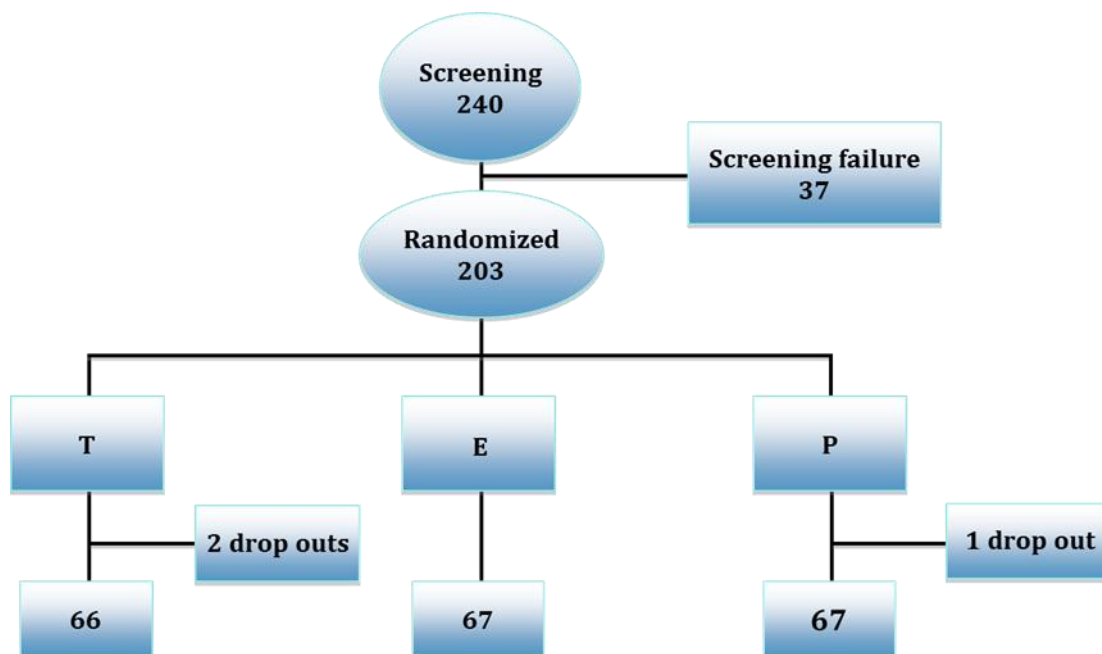
International Neuropsychiatric Interview (MINI) was performed by a psychiatrist. Eleven women fulfilled the criteria. One woman discontinued the study after the first PET investigation due to private reasons and was not included in the analyses.

The study in paper *I*, *II* and *III* was approved by the local Ethics Committee in Stockholm (2006/481-31/3) and the Swedish Medical Products Agency (151:2006/29773), and all women gave their written consent to participate in the study.

The study in paper *IV* was approved by the local Ethics Committee in Stockholm (04-850/2) and the Swedish Medical Products Agency (2004-002677-24).

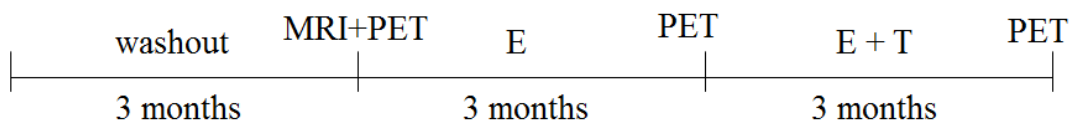
### 3.2 STUDY DESIGN

In paper *I*, *II* and *III*, women were randomized to one out of three treatment groups. Either estrogen (Estradiol valerate, 2 mg/day (E)), testosterone (Testosterone undecanoate, 40 mg/day (T)) or placebo treatment (P) for a period of four weeks was given (Fig 7). Randomization was carried out with blocks of 4 and 12 subjects in each block. The study medication was tested and manufactured by the National Corporation of Swedish Pharmacies in identical capsules. At baseline visit, blood samples were collected and study medication was given. After one month of treatment, the first morning after the cessation of therapy, a new blood sample was taken after an overnight fast. After receiving breakfast at the clinic, each woman (in groups of 1–6 individuals) participated in the experimental session that took approximately 1.5 h. All experimental sessions were conducted by the same person, between 08.45 and 10.15 a.m. The study was carried out between March 2007 and June 2008.



**Figure 7.** The design of the double-blind randomized trial.

In paper *IV*, each subject received three months treatment with transdermal estradiol 100  $\mu\text{g}/24$  hours (E) followed by three months of oral testosterone addition with testosterone undecanoate 40 mg daily (E + T). Investigations were performed before and after three and six months of treatment, respectively. At baseline, a blood sample was collected before the participant underwent magnetic resonance imaging (MRI), PET and cognitive tests. After three and six months of treatment, the same investigations were performed except for the MRI.

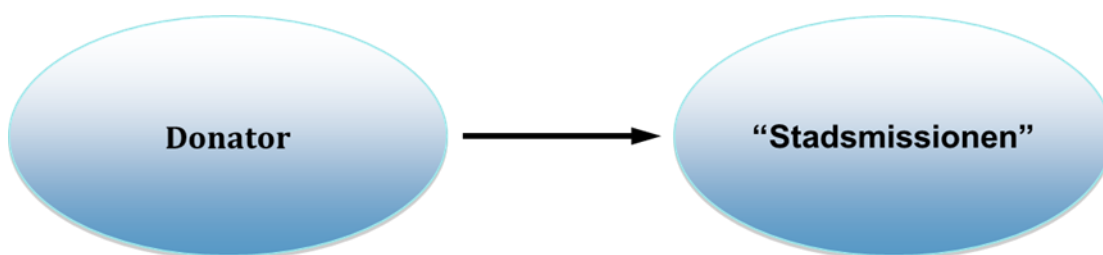


**Figure 8.** The design of the PET study.

### 3.3 ECONOMIC BEHAVIOR

In paper *I*, subjects participated in economic experiments. The monetary stakes in the experiments were sizeable, and on average a subject earned SEK 1.050 (approximately \$150; exchange rate at the time of the study; \$1=SEK 6.5). In some of the experiments a subject was randomly matched with another anonymous subject. Subjects were never matched with the same counterpart more than once.

A modified dictator game was used to measure altruistic behavior (Eckel and Grossman 1996). Each subject decided how to allocate SEK 200 between herself and a charitable organization (a charity called “Stadsmissionen,” which predominantly focuses on helping the homeless in Sweden). The size of the donation is our measure of *altruism* (Fig 9).



**Figure 9.** Altruism: donator decides how to allocate SEK 200 between herself and a charitable organization. The size of donation is a measure of altruism.

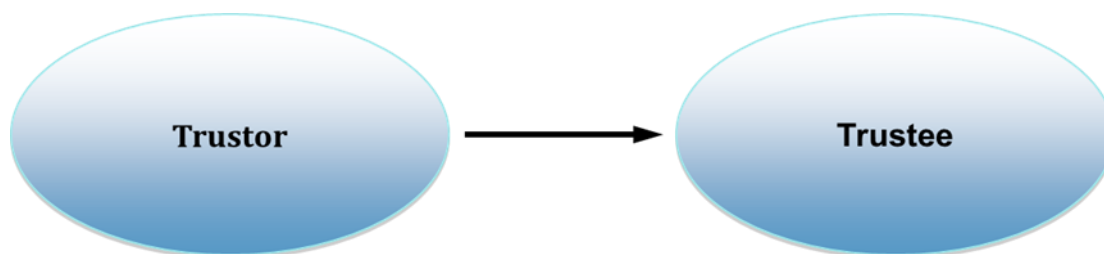
The second and third experiment concerned ultimatum game behavior (Güth et al 1982). The ultimatum game is a 2-person game in which one subject proposes how to split a sum of money and the other subject can accept or reject the proposal. If the proposal is accepted, the money is split according to the proposal; otherwise, neither subject gets any money.

In the proposer role (second experiment), subjects propose a division of SEK 400 between themselves and an anonymous counterpart (only proposals in even SEK 50

increments were allowed). Because 92% of the subjects proposed a 50/50 split, there is no scope for finding an effect on sex hormones on proposals in the ultimatum game.

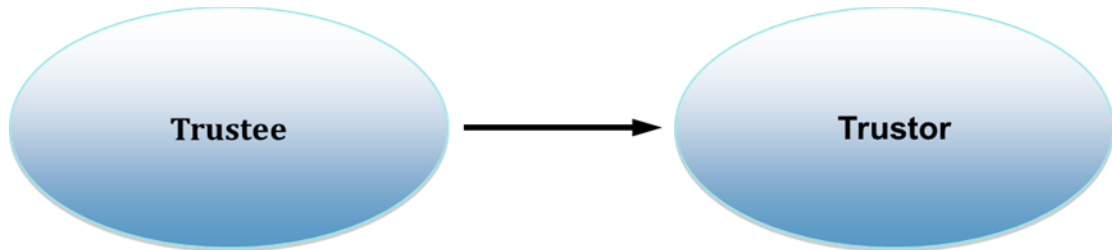
In the third experiment, subjects played the role of an ultimatum game responder. We used the so-called strategy method (Camerer 2003) to elicit the acceptance threshold in the ultimatum game, with each subject determining whether she would accept or reject every possible proposal (in multiples of SEK 50) before learning the actual proposal. The acceptance threshold for each individual is defined as the midpoint of the lowest offer accepted and the previous offer. All subjects exhibited monotonic acceptance behavior in the range of offers between 0% and 50%. The acceptance threshold is our measure of *reciprocal fairness*.

The fourth and fifth experiment concerned trust game behavior (Berg et al 1995). A trust game is a 2-player game in which one player, the trustor, decides how much of an endowment to invest. The investment is multiplied by 3, where on the other player, the trustee, decides how to allocate the resulting amount between herself and the trustor. In the fourth experiment, each subject played the trustor role, deciding how much money out of an initial endowment of SEK 150 to send to a randomly selected anonymous counterpart. In the fifth experiment, each subject played the role of trustee, deciding how much to send back to the investor for every possible investment (SEK 50, 100, and 150), before learning the actual investment. The investment in the first stage is our measure of *trust* (Fig 10).



**Figure 10.** Trustor decides how much of SEK 150 to invest in the trustee. Investment is then multiplied by 3. The trustee decides how to allocate the resulting amount between herself and the trustor. The investment is our measure of trust.

The average back transfer (in SEK) for the three possible investment levels is our measure of *trustworthiness* (Fig 11).



**Figure 11.** Trustee decides how much of the investment (multiplied by 3) to send back to the investor (trustor). The back transfer (in SEK) is our measure of trustworthiness.

The final experiment with real monetary stakes measured the subjects risk aversion (Holt and Laury 2002). Each subject made six choices between a certain payoff and a 50/50 gamble to win SEK 400. The certain payoffs were set to SEK 80, 120, 160, 200, 240, and 300. After the subjects had made their six choices, one of the choices was randomly chosen for payoff by rolling a die. The gamble was resolved by a coin toss in front of the subjects. The experiment determines seven intervals for the certainty equivalent and the certainty equivalent was set to the midpoint of the interval. All subjects made monotonic choices. The certainty equivalent is our measure of *risk aversion*.

The subjects also filled out a questionnaire with two hypothetical questions about risk attitudes. The first question asks the subject to imagine that she has won SEK 1 million on the lottery and that she can invest some of this money in a risky asset with an equal probability of doubling the investment and losing half the investment (Dohmen et al 2005). Subjects can choose between six levels of investments: SEK 0, 200.000, 400.000, 600.000, 800.000, and 1.000.000. The investment is our measure of *risk investment*. The second question measures general risk attitudes on a 0 to 10 scale, where 0 is complete unwillingness to take risks and 10 is complete willingness to take risks (Dohmen et al 2005). The scale value is our measure of *risk assessment*.

### 3.4 COGNITIVE FUNCTION

In paper *II*, cognitive function was investigated by verbal fluency test, verbal memory test and spatial ability test.

In the verbal fluency test, each woman was asked to write as many words as possible beginning with a specified letter during 1 minute (Lezak 1995). The letters were F, A, S, and N, each on a separate piece of paper and one at a time. The sum of words generated from the four letters was taken as measure of *verbal fluency*.

In the test of episodic verbal memory, a list of 12 common unrelated nouns were presented verbally with the instruction to remember as many of the words as possible for an immediate free recall test (Herlitz et al 1997). Four lists were presented one at a time. The sum of nouns remembered from the four lists (range, 0–48) was taken as measure of *verbal memory*.

Spatial ability was measured by using a mental rotation test developed by (Vandenberg and Kuse 1978) based on (Shepard and Metzler 1971). The test is a paper-and-pencil test containing 20 items. In each item the woman is instructed to select two figures from four alternatives that are rotated versions of the target figure to the left. Two points are given for a correct answer on two, one point for selecting one correct and zero otherwise. The time limit is 10 minutes. The score on the mental rotation test (range, 0–40) was taken as measure of *spatial ability*.

In paper *IV*, we used Beck Depression Inventory (BDI) (Beck et al 1996) which is a multiple-choice self-report inventory for measuring *symptoms of depression*. It contains 21 questions, each answer being scored on a scale value of 0 to 3. Higher total scores indicate more severe depressive symptoms. The cutoffs are: 0–13: minimal depression; 14–19: mild depression; 20–28: moderate depression; and 29–63: severe depression.

Beck Anxiety Inventory (BAI) is a 21-question multiple-choice self-report inventory that is used for measuring *anxiety* (Beck et al 1993). BAI has a maximum score of 63, the higher the score is the more anxiety a person is suffering from.



As a measure of *speed of processing* we used the Trial Making Test part A (TMT-A) (Lezak 1995). The participant was asked to draw a line between numbers in ascending order as fast and as accurate as possible without lifting the pen. The dependent variable is time to completion measured in seconds.

To estimate the capacity to plan and execute ones actions we used the Trial Making Test part B (TMT-B) as a measure of *executive functions* (Lezak 1995). As in part A, the task is to draw a line as fast and as accurate as possible, however in part B of the test, the series alternates between numbers in ascending order and letters in alphabetic orders (i.e. 1, A, 2, B, 3, C ... etc.). Because of this small change in the task the participant needs more to rely on a strategy where one subdivides the task into subordinate goals and execute them in the right order. Because of this feature TMT-B is believed to recruit more executive functions than TMT-A. The dependent variable is time to completion measured in seconds.

*Verbal fluency* was estimated by the same fluency test as in paper II (Lezak 1995). For *category fluency* three categories were tested: animals, fruits, and vegetables, with a time limit of 60 seconds for each (Lezak 1995)

As a measurement of *social cognition*, the test reading the mind of the eyes was used (Baron-Cohen et al 2005). In the test, the participant was presented with 36 pictures showing only the eyes of a person and the participant was given four words. The instruction was that the participant should match one of the four words that the participant thinks best reflects the particular mental state the person on the picture is in. The dependent variable in the test was the number of correct answers out of 36.

### 3.5 POSITRON EMISSION TOMOGRAPHY (PET)

*PET* is a nuclear medicine medical imaging technique (Fig 12). The system detects pairs of gamma rays emitted indirectly by a positron-emitting radioisotope, which is introduced into the body on a metabolically active molecule. Using PET pre- and postsynaptic receptor density, affinity, neurotransmitter release, enzyme activity and drug delivery and uptake are possible to quantify with high selectivity and sensitivity of a pico-to nan-molar range (Jovanovic et al 2006). Images of metabolic activity in space are then reconstructed by computer analysis.

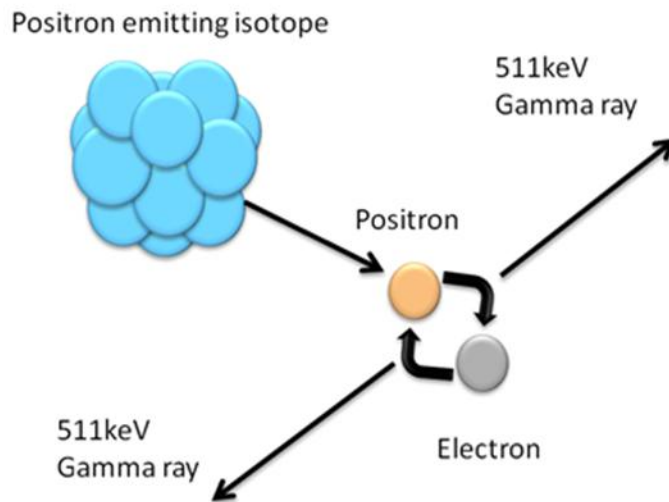


**Figure 12.** PET camera at PET centrum, Karolinska University Hospital.

The principles of PET (Fig 13):

1. The radioactive tracer, a small quantity of a ligand labeled with positron emitter, e.g.  $[^{11}\text{C}]$ , is injected into the body.
2. The positron-emitting radionuclide ejects a positron ( $+\beta$ ) from the nucleus as it decays.
3. The positron will combine with an electron in the tissue and annihilate.
4. The annihilation releases energy and results in conversion of the electron and positron into a pair of 511 keV gamma emitted in opposite directions.
5. Two gamma rays, traveling 180 apart are detected in coincidence.
6. The pair of photons produced from a single annihilation will register on opposing pairs of scintillation detectors as a “coincidence event”.

7. Tomographic technique analyzes this detection to yield images of the distribution of the administered positron-emitting radiotracers (Joffe et al 2006).



**Figure 13.** The principles of PET.

The development of PET and selective radioligands has paved the way for studies on the expression of serotonin and 5-HTT proteins in human brain *in vivo*. Several high affinity radioligands have been developed for the examination of serotonin 1A (Pike et al 1996) and serotonin 2A (Ito et al 1998) receptors over the years, as well as the 5-HTT (Houle et al 2000, Lundberg et al 2005). The methodological development in this field facilitated the research on the status and distribution of serotonin receptors and 5-HTT binding potentials (BP) in healthy humans and in individuals with psychiatric disorders.

[<sup>11</sup>C]MADAM, N,N-dimethyl-2-(2-amino-4-methylphenylthio) benzylamine is a radioligand with high specificity and selectivity for the 5-HTT which was developed at the laboratory at the PET center, Karolinska University Hospital (Halldin et al 2005). A radioligand developed earlier for 5-HTT, the isoquinolin derivate <sup>11</sup>C-(+)-6β-(4-methylthiophenyl)-1,2,3,5,6 α, 10β-hexahydropyrrolol[[2,1-a]isoquinoline ([<sup>11</sup>C]McN 5652) has been used to image 5-HTT in non human primates and humans (Suehiro et al 1993, Szabo et al 1995).

Another radioligand that was often used for 5-HTT is dyphenil sulfide derivate 11C-3-amino-4-(2-dimethylaminomethylphenylthio)-benzonitrile [11C]-DASB, which has shown a higher signal to noise ratio compared to [11C]McN 5652 (Frankle et al 2004). Lundberg and collaborators (2006) compared the data from their study with those of Frankle et al (2004) and found even higher signal –to noise ratio for [11C]MADAM when compared to [11C]–DASB. Test-retest measurement of [11C]MADAM binding to the 5-HTT has shown good to excellent reliability by using the simplified reference tissue model (SRTM) (Lundberg et al 2005, Jovanovic et al 2008) suggesting [11C]MADAM as highly suitable for clinical studies, such as the study in paper IV.

[11C]MADAM was prepared as described previously (Halldin et al 2005). Each subject was placed recumbent with her head in the PET system. The radioligand was injected into the left antecubital vein during 2 sec and the cannula was immediately flushed with 10 ml saline.

A mean sterile phosphate buffer solution of 210 MBq (SD = 37.5) (PET 1) and 205 MBq (SD = 29.3) (PET 2) and 209 MBq (SD = 18.9) (PET 3) of [11C]MADAM was injected intravenously. Brain radioactivity was measured during 93 min for [11C]MADAM (frame sequence: 3 x 1, 4 x 3, 9 x 6 and 3 x 1, 4 x 3, 13 x 6 min, respectively).

*MRI scans* were performed on a 1.5 T GE Signa system (Milwaukee, WI) using a 3-dimensional (3-D) spoiled gradient recalled sequence (a standard spin-echo sequence with a 256 x 256 matrix; repetition time of 4 sec). Proton density (17 msec) and T2 - weighted images (85 msec) were obtained to achieve one set of images with high spatial resolution and another with high sensitivity for pathology.

The PET images were acquired using an ECAT. Exact HR 47 scanner (CTI/ Siemens, Knoxville, TN) run in 3D mode (resolution in plane = 3.8 mm; the axial resolution =4.0 mm full width half maximum (Wienhard et al 1994). A head fixation system with an individual plaster helmet was used both in the PET and MRI measurements to allow the same head positioning in the two imaging modalities and between scans (Bergström et al 1981). After acquisition, the MRI and PET datasets were coregistrated according to the procedure described by Jovanovic (2008).

*Regions of Interest* (ROI) included cortical (lateral frontal cortex, medial frontal cortex, orbitofrontal cortex, lateral temporal cortex, medial temporal cortex, lateral and medial parietal cortex, sensory motor cortex, occipital cortex and insular cortex), limbic (anterior and posterior cingulate, amygdala, hippocampus, and parahippocampal gyrus), and sub-cortical regions (caudate, putamen, thalamus, globus pallidus, and raphe). ROI were outlined using the automated delineation method (anatomical atlas implemented in the SPM2 software) (Tzourio-Mazoyer et al 2002), with exception of the dorsal raphe ROI which was manually delineated (6 mm -diameter circular ROI) and centred over the raphe nuclei evident by the highest area of focal tracer uptake of [11C]MADAM on summated PET images in four horizontal sections. The cerebellar cortex was used as a reference tissue for the quantification of 5-HTT BP.

The BP was estimated using the simplified reference tissue model (SRTM), which has been validated for [11C]MADAM (Lundberg et al 2005). SRTM is a non-invasive modeling approach that uses cerebellar time activity curve (TAC) as an indirect input function for the calculations of BP. The utilization of cerebellum as a reference tissue in SRTM was based on the assumption of the virtual absence of radio ligand binding in this region. For the 5-HTT, in vitro studies have demonstrated negligible densities of both the transporter in the cerebellum, which should not account for specific binding (Cortes et al 1988, Hall et al 1997). With regard to radioligand [11C]MADAM, in monkeys pretreatment with citalopram decreases [11C]MADAM binding to the level of cerebellar TAC in the regions with higher serotonin transporter density (Halldin et al 2005). However, the possibility of a few percent activity in the cerebellum cannot be excluded and autoradiographic examination of the human postmortem cerebellum suggested higher concentrations of the 5-HTT in the cerebellar vermis compared to cerebellar grey matter (Parsey et al 2005). The cerebellar vermis was accordingly excluded from the cerebellar ROI analysis.

The SRTM accounts for regional differences in the influx rate constant ( $K_1$ ) between the ROI and the cerebellum as well as regional differences in the time course of free and non-specifically bound radio ligand. The approach provides with the parameter referred to as BP and was calculated according to the formula:  $BP = (k_3/k_4) = B_{max} f_2 / (K_d [1 + \sum_i F_i / K_{di}])$  where  $k_3$  and  $k_4$  refer to the exchange of tracer between the free and a specifically bound compartment,  $B_{max}$  the density of receptor,  $f_2$  is the "free fraction" of unbound radio ligand in the tissue,  $K_d$  the dissociation constant for the

radio ligand, and  $F_i$  and  $K_{di}$  are the free concentration and dissociation constant of the competing endogenous ligand.

### 3.6 HORMONE ANALYSES

In paper *I*, *II* and *III*, serum concentrations of testosterone and estradiol were determined by radioimmunoassay by using commercial kits from Diagnostic Products Corporation (Coat-a-Count, testosterone), and from Orion Diagnostica (Spectria, estradiol). Serum concentrations of SHBG and FSH were determined by chemiluminescent enzyme immunometric assays (IMMULITE, Diagnostic Products). Detection limits and within and between assay coefficients of variation were 0.2 nmol/L, 6% and 11% for testosterone; 5 pmol/L, 7% and 10% for estradiol; 0.2 nmol/L, 7% and 13% for SHBG; and 0.1 IU/L, 5% and 8% for FSH. Apparent concentrations of free testosterone were calculated from values of total testosterone, SHBG, and a fixed albumin concentration of 40 g/L by successive approximation with a computer program based on an equation system derived from the law of mass action (Södergård et al 1982).

In paper *III*, serum ADG was determined by Beckman Coulter Inc, Fullerton, CA. Serum IGF-I was determined by direct chemiluminiscence enzyme immunoassay using commercial kits from Siemens Medical Solutions., Los Angeles, CA, USA (Immulate®). After solid phase extraction on Sep-Pak C18 micro columns according to a protocol given by the manufacturer of the assay kit, serum oxytocin was determined by enzyme immunoassay using a commercial kit obtained from Assay Designs, Inc, Ann Arbor, MI.

Detection limits and within and between assay coefficients of variation were 0.85 nmol/L, 10% and 9% for ADG, 20 µg/L, 3.6% and 6.6% for IGF-I, and 11.7 ng/L, 8.9% and 9,5 % for oxytocin, respectively.

In paper *IV*, serum concentrations of estradiol and total testosterone were determined by radioimmunoassay using commercial kits from Orion Diagnostica Oy Espoo Finland (Spectria, estradiol, Spectria, testosterone). Serum concentrations of SHBG were determined by chemiluminescent enzyme immunometric assays (IMMULITE

Siemens). Detection limits and within and between assay coefficients of variation were for estradiol 5 pmol/L, 7% and 10%; for testosterone 0.11 nmol/L 5.3% and 5.4% and for SHBG 0.2 nmol/L 6.5% and 8.7%. The apparent concentrations of free T were calculated as in paper *I*, *II* and *III*.

### 3.7 STATISTICAL ANALYSIS

In paper *I*, data are presented as mean and 95% confidence interval (CI). Differences between the three treatment groups for any of the studied economic behaviors were tested by Mann–Whitney U test. Correlations were assessed by Spearman rank correlation test.

In paper *II*, normally distributed data are presented as mean  $\pm$  standard deviation (SD) or 95% CI, otherwise as median and quartile range (Q25-75). Differences in cognitive abilities between treatment groups were analyzed using one-way analysis of variance (ANOVA) and one way analysis of covariance (ANCOVA), with age as the covariate. Kruskal-Wallis ANOVA by ranks followed by multiple comparisons between treatment groups was performed for hormone data.

In paper *III*, comparison between groups was performed by ANOVA and within groups by Wilcoxon signed rank test. Correlations between hormone levels and cognition during treatment were assessed using Spearman rank correlation test.

In paper *IV*, values are presented as mean  $\pm$  SD or median and quartile range (Q25-Q75) according to distribution. The differences between the three treatment regimens for each PET-variable were analyzed using procedure Mixed in SAS®. We computed a mixed model with Treatment (no treatment, E and E + T) as the within-subjects variables. In case of a significant treatment effect, pairwise comparisons between treatment means were performed. The Spearman rank order correlation coefficient was used to measure the association between the PET-variables and hormones levels, and cognitive abilities, respectively.

For all papers  $p < 0.05$  was considered statistically significant.

## 4 RESULTS

### 4.1 OUTCOME OF THE RANDOMIZED TRIAL (I, II, III)

Some baseline characteristics for the 200 postmenopausal women who completed the randomized trial (I-III) are given in Table I. The treatment groups were quite comparable with regard to age, BMI and natural menopause, time since menopause, previous use of HRT, duration and time since cessation of HRT and level of education. Mean level of menopausal years was around 7-8 years and there was generally a substantial “wash out” period after cessation of HRT. However, the mean value for age among women in the estrogen group was about one year higher than for those treated with placebo. The proportion of women with higher education was somewhat larger in the testosterone group but this numerical difference was not significant.

**Table I.** Baseline characteristics of the 200 postmenopausal women who completed the randomized clinical trial (I-III) and were treated with testosterone, estrogen, or placebo (mean  $\pm$  SD or median and quartile range [Q25–Q75]).

	<b>Testosterone</b> (n = 67)	<b>Estrogen</b> (n = 66)	<b>Placebo</b> (n = 67)
Age, years	58.3 $\pm$ 4.2	59.7 $\pm$ 3.7*	58.1 $\pm$ 4.0
BMI, kg/m <sup>2</sup>	24.3 $\pm$ 2.6	24.5 $\pm$ 2.7	24.0 $\pm$ 2.8
Menopausal age, years	51.9 $\pm$ 3.1	52.0 $\pm$ 3.8	51.5 $\pm$ 3.2
Years since menopause	7.4 $\pm$ 4.2	8.7 $\pm$ 4.3	7.5 $\pm$ 4.2
Previous HRT, %	63	58	49
Duration of HRT, years	2.5 (1.0-5.0)	3.0 (1.0-8.0)	2.0 (1.0-5.5)
Time since end of HRT, years	3.5 (2.0-6.0)	4.0 (2.0-6.0)	3.2 (1.0-5.0)
Higher education, %	71	63	62



### 4.1.1 Hormone levels

As illustrated in Table II, serum concentrations of estradiol and total and free testosterone increased significantly after four weeks of treatment compared to placebo. Mean total and free testosterone levels were about 3 and 4.5 fold higher after treatment than baseline values in the testosterone group. In the estrogen group, the mean estradiol level was increased about 10 fold after four weeks of treatment.

**Table II.** Serum levels of total and free testosterone and estradiol at baseline and after four weeks of treatment (mean  $\pm$  SD or median and quartile range [Q25–Q75]).

	<b>Testosterone</b> (n = 67)	<b>Estrogen</b> (n = 66)	<b>Placebo</b> (n = 67)
<b>Testosterone</b>			
<b>(nmol/L)</b>			
baseline	0.52 $\pm$ 0.31	0.56 $\pm$ 0.33	0.53 $\pm$ 0.37
4 weeks	2.31 $\pm$ 1.85***	0.52 $\pm$ 0.27	0.58 $\pm$ 0.39
<b>Free Testosterone</b>			
<b>(pmol/L)</b>			
baseline	7.2 $\pm$ 5.3	8.0 $\pm$ 5.4	7.7 $\pm$ 5.9
4 weeks	40.0 $\pm$ 35.0***	4.7 $\pm$ 3.0***	8.3 $\pm$ 6.2
<b>Estradiol</b>			
<b>(pmol/L)</b>			
baseline	22.1 (14.2–35.8)	21.8 (16.2–28.4)	22.4 (11.3–31.2)
4 weeks	28 (15 - 41)**	225 (157 - 283)***	24 (13 - 32)

### 4.1.2 Economic behavior

After four weeks of treatment with either 2 mg of estradiol, 40 mg of testosterone undecanoate or placebo all women participated in total of seven different economic experiments (*I*). There were no apparent differences between the groups in their performance at none of these economic tests (Table III).

**Table III.** Mean values and 95% CI for outcome in the different economic experiments according to treatment groups.

	<b>Testosterone</b> (n = 67)	<b>Estrogen</b> (n = 66)	<b>Placebo</b> (n = 67)
<b>Altruism</b> max donation 200 SEK	107 [88-126]	118 [99-137]	107 [87-127]
<b>Reciprocal fairness</b> max acceptance threshold 200 SEK	134 [119-149]	141 [127-155]	138 [124-153]
<b>Trust</b> max acceptance threshold 450 SEK	117 [108-127]	104 [92-116]	113 [104 -123]
<b>Trustworthiness</b> max acceptance threshold 450 SEK	423 [386-460]	392 [368-417]	389 [361-416]
<b>Risk aversion</b> winning max 400SEK	192 [171-214]	195 [175-214]	203 [183-223]
<b>Risk investment</b> max investment 1000 000 SEK	376 [327-425]	367 [308-425]	355 [294-416]
<b>Risk assessment</b> scale 0-10	5.4 [5.0-5.9]	5.1 [4.6-5.6]	5.2 [4.8-5.7]

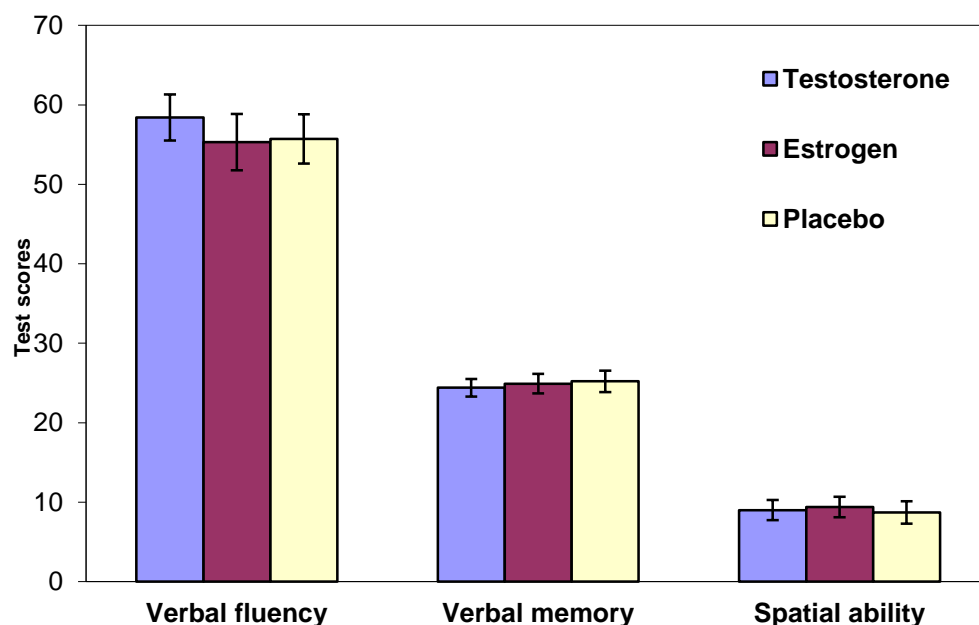
Contrary to our hypothesis, there were no significant differences in *altruism* (the amount donated to charity in the dictator game), *reciprocal fairness* (the acceptance threshold in the ultimatum game), *trust* (the amount of money invested in the trust game), and *trustworthiness* (the back transfer in the trust game). Also mean values for *risk aversion* (the certainty equivalent to a 50/50 gamble to win SEK 400), *risk investment* (the amount of money hypothetically invested in a profitable but risky

investment), and *risk assessment* (the general willingness to take risks on a 0-10 scale) were quite similar between the groups.

There was an overall tendency among the women to accept an approximate 50/50 split of the money and risks allocated for the different experiments. Also the 95% CI were mostly quite small. The apparent homogeneity within the groups clearly would hamper the detection of a possible hormonal influence. There was considerable heterogeneity in the serum hormone levels both for the increase of testosterone in the testosterone group (range, 0-10.15 ± 1.84 nmol/L) and for the increase of estradiol in the estrogen group (range, -1-594.5 ± 103.5 pmol/L). Therefore correlations were tested between the size of the increase of the respective hormone and economic behavior. However none of these correlations were significant ( $p > 0.05$ ).

#### 4.1.3 Cognitive function

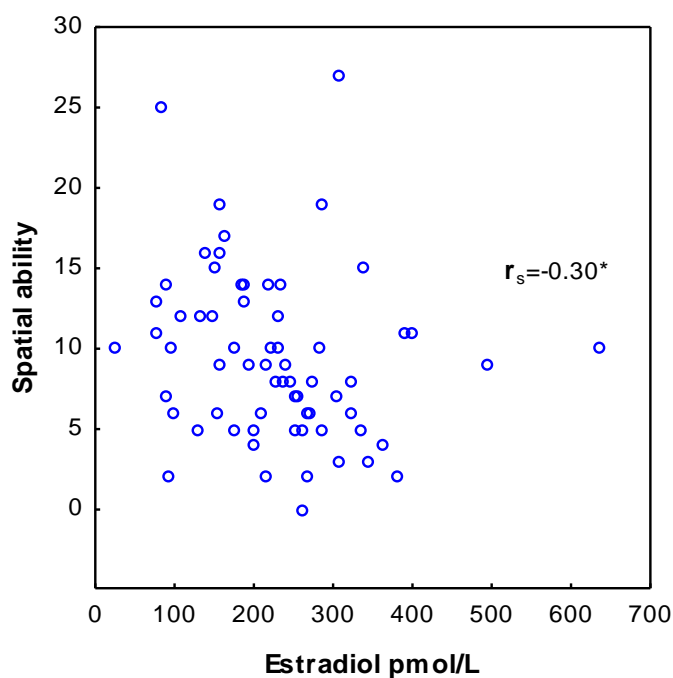
After four weeks of hormonal treatment the women also underwent a variety of tests to assess cognitive function (*II*). Again there were no significant differences between the three treatment groups in verbal fluency, verbal memory, or spatial ability ( $p > 0.05$ ).



**Figure 14.** Mean values and 95% CI of verbal fluency, verbal memory, and spatial ability after four weeks of treatment with testosterone, estrogen, and placebo. There were no significant differences between any of the treatment groups ( $p > 0.05$ ).

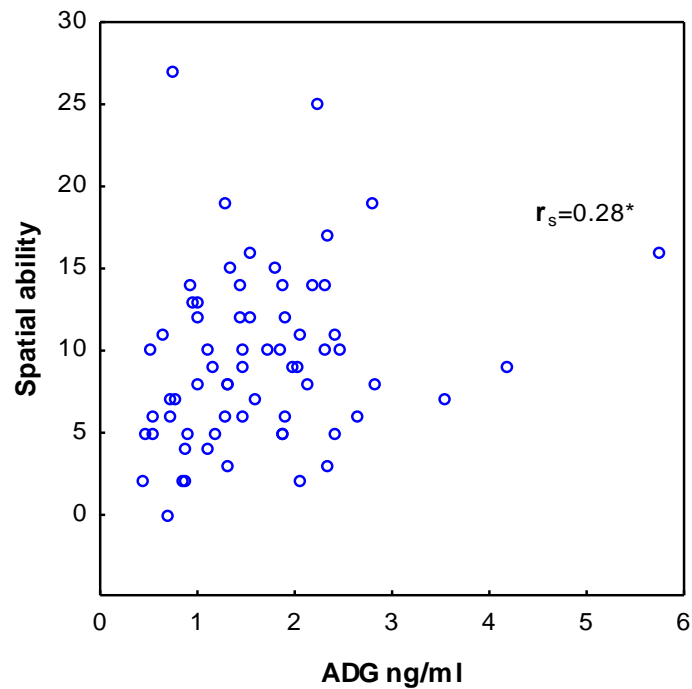
#### 4.1.4 Sex hormone levels in association with cognitive function

Although there were no significant differences between the three treatment groups in verbal memory, verbal fluency or spatial ability, there were several significant correlations between sex hormone levels and some aspects of cognitive function in the postmenopausal women. High absolute estradiol levels, a high estrogen/testosterone ratio and increasing estradiol levels during estrogen treatment were all associated with lower spatial ability (Fig 15).



**Figure 15.** Correlations between estradiol levels and spatial ability in postmenopausal women treated with estrogen.

On the other hand, serum levels of the testosterone metabolite, ADG, were positively associated with spatial ability (Fig 16). This association was further enhanced in the subgroup of women with low range of estradiol values ( $r_s = 0.40$ ,  $p < 0.05$ ).



**Figure 16.** Correlations between ADG levels and spatial ability in postmenopausal women treated with estrogen.

Furthermore, our results showed a negative relationship between testosterone and verbal fluency ( $r_s = -0.27$ ,  $p < 0.05$ ), and a positive association between the estradiol/testosterone ratio and verbal fluency ( $r_s = 0.25$ ,  $p < 0.05$ ) in the placebo group. There was also a strong negative correlation between IGF-I and verbal fluency in those women with high estradiol levels after estrogen treatment ( $r_s = -0.57$ ,  $p < 0.01$ ). Verbal memory showed a positive association with ADG in the whole group of women treated with estrogen and in those with high estrogen levels ( $r_s = 0.40$ ,  $p < 0.01$ ).

## 4.2 OUTCOME OF THE PET STUDY

In paper IV, ten women completed the study. One woman discontinued due to personal reasons. Baseline characteristics of the ten healthy surgically postmenopausal women are listed in Table IV.

**Table IV.** Baseline characteristics of the surgically postmenopausal women are presented as mean  $\pm$  SD or median and quartile range [Q25–Q75]).

---

	n = 10
Age, years	58.4 $\pm$ 4.7
BMI, kg/m <sup>2</sup>	25.9 (24.0-27.7)
Time since oophorectomy, years	7.6 $\pm$ 4.0
Previous HRT, %	60
Duration of HRT after surgery, years	1 (0-3.3)
Higher education, %	40

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### 4.2.1 Hormone levels

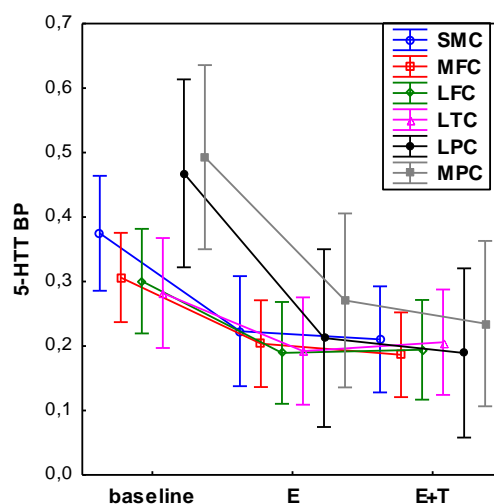
Serum levels of hormones at baseline, after three months of treatment with transdermal estradiol 100 $\mu$ g/day and after another three months of combined treatment with estradiol and oral testosterone undecanoate 40mg/day are given in Table V. After three months of estrogen treatment, mean serum levels of estradiol corresponded to values in the early follicular phase of menstruating women but there was also a considerable individual variation. As expected, SHBG concentrations increased by estrogen treatment and as consequence levels of free testosterone were significantly reduced. After combined estrogen and testosterone treatment, the mean level of estradiol was further enhanced and total and free testosterone levels significantly increased.

**Table V.** Serum levels of hormones in healthy surgically postmenopausal women at baseline, after three months of treatment with estrogen and after another three months of treatment with estrogen and testosterone in combination (mean  $\pm$  SD).

	Baseline	Estrogen	Estrogen + Testosterone
Estradiol, pmol/l	23 $\pm$ 8	228 $\pm$ 138 <sup>a***</sup>	280 $\pm$ 151 <sup>b***</sup>
Testosterone, nmol/l	1.07 $\pm$ 0.43	0.90 $\pm$ 0.35	1.71 $\pm$ 1.06 <sup>b*c*</sup>
Free testosterone, pmol/l	15.4 $\pm$ 6.6	10.6 $\pm$ 5.0 <sup>a**</sup>	23.8 $\pm$ 15.4 <sup>b*c**</sup>

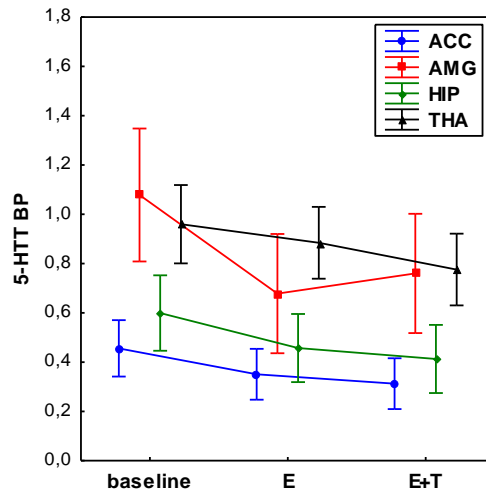
#### 4.2.2 5-HTT binding potential

Both hormonal therapies had significant effects on the 5-HTT BP in several brain regions as measured by PET with the [11C] MADAM ligand. There was a significant decrease in 5-HTT BP in several cortical regions (sensory motor cortex, medial frontal cortex, lateral frontal cortex, lateral temporal cortex, lateral parietal cortex, medial parietal cortex, lateral occipital cortex) (Fig 17).



**Figure 17.** 5-HTT BP in different cortical areas at baseline, after three months of treatment with estrogen (E) and after another three months of treatment with estrogen and testosterone in combination (E+T). SMC = sensory motor cortex, MFC = medial frontal cortex, LFC = lateral frontal cortex, LTC = lateral temporal cortex, LPC = lateral parietal cortex, MPC = medial parietal cortex.

Effects of hormonal treatment were also pronounced within the limbic system e.g. amygdala and in the parahippocampus (Fig 18). However, there was no significant difference in values of 5-HTT BP between the two treatment periods in any of the investigated brain areas.



**Figure 18.** 5-HTT BP in different limbic areas at baseline, after three months of treatment with estrogen (E) and after another three months of treatment with estrogen and testosterone in combination (E+T). ACC = anterior cingulate cortex, AMG = amygdala, HIP = hippocampus, THA = thalamus.

The scores of BDI were significantly decreased after estrogen treatment compared to baseline ( $6.0 \pm 6.0$  vs  $6.8 \pm 5.9$ ,  $p < 0.05$ ). The levels were also lower after combined treatment but did not reach significance. There was no significant change in BAI, TMT-A, TMT-B or social cognition. However, both letter fluency and category fluency increased significantly after combined treatment with estrogen and testosterone compared to baseline ( $54.0 \pm 13.1$  vs  $44.6 \pm 10.2$ ,  $p < 0.05$ , and  $69.8 \pm 9.8$  vs  $54.2 \pm 9.1$ ,  $p < 0.01$ ).



## 5 DISCUSSION

### 5.1 SEX HORMONES AND ECONOMIC BEHAVIOR

The results from our randomized trial give no support for testosterone or estrogen treatment having any substantial effect on economic behavior in healthy postmenopausal women.

There were only a few previous studies, which suggested that endogenous testosterone levels could have an influence on economic behavior. Most of these studies have been performed in men (Burnham 2007, Apicella et al 2008). Testosterone levels have been associated with increased behavior of competition and dominance (Archer 2006) and reduced fear (van Honk et al 2004, Hermans et al 2006). Also the evaluation of the risk balance between punishment and reward may be influenced (Takahashi et al 2006). Furthermore, endogenous testosterone has been associated with high risk behavior e.g. gambling and alcohol use (Mazur 1995, Blanco et al 2001). In a study of traders from the City of London, daily profits were found to be positively correlated with their levels of salivary testosterone (Coates and Herbert 2008). Also the level of average profitability was negatively correlated with the ratio between the length of the second and fourth finger (Coates and Herbert 2008). A lower finger ratio (2D:4D ratio) is believed to reflect higher fetal exposure to testosterone (Manning et al 2002). Another study reported that low 2D:4D ratio in men was associated with higher risk taking and higher scores in abstract reasoning ability (Branas-Garza and Rustichini 2011).

Relationships between endogenous sex hormone levels and risk behavior in women have also been reported. Thus, naturally cycling women demonstrated reduced risk activities during the ovulatory phase (high estradiol levels) compared to other phases of the menstrual cycle (Chavanne and Gallup 1998, Bröder and Hohmann 2003). However, correlation studies do not prove causality between sex hormones and economic behavior.

To our knowledge, the present study (*I*) is the first placebo-controlled trial to investigate the effect of testosterone and estrogen treatment on economic behavior in postmenopausal women. The interpretation of our negative results is not clear, but certainly the respective treatment doses and resulting hormone levels may be of importance. Also factors like women's age and the duration of treatment might have influenced the results.

It is well known that similar doses of estrogen are effective for treatment of menopausal symptoms e.g. flushing, sweating, and sleep disorder (Santen et al 2010). Also, testosterone therapy resulting in similar serum levels as in this study has been shown to improve psychosexual function, e.g. arousal, desire, satisfaction, and well-being in postmenopausal women (Shifren et al 2000, Flöter et al 2002, Davis et al 2008).

The increase of total testosterone levels in the present study after four weeks treatment of postmenopausal women was about four-fold and reached a level around 2 nmol/L. In comparison, a single supraphysiologic dose of testosterone resulting in a 10-fold increase of the circulating level was reported to enhance risk behavior in economic experiments (van Honk et al 2004) and impair cognitive empathy in young healthy women (van Honk et al 2011). Thus, there is some data to support an effect of testosterone treatment on economic behavior in women, at least in those of younger age (Eisenegger et al 2010, van Honk et al 2012).

Interestingly, a recent study showed that a single dose of testosterone caused a substantial increase in fair bargaining behavior in young healthy women (Eisenegger et al 2010, van Honk et al 2012). Moreover, van Honk and colleagues (2012) demonstrated increased social cooperation after testosterone administration in female students with low levels of prenatal testosterone, estimated by the right hand's 2D:4D digit ratio. This is in clear contrast to the hypothesis that testosterone is linked to antisocial and egoistic behavior in humans. Obviously, there is a great need of increased knowledge about the role of sex hormones in human behavior.

## 5.2 INFLUENCE OF SEX HORMONES ON COGNITIVE FUNCTION

We performed a randomized controlled trial (*II*) where no significant effects were found between treatment with estrogen, testosterone and placebo on verbal memory, verbal fluency or spatial ability in healthy postmenopausal women.

Investigations of HRT and cognition in postmenopausal women have yielded inconsistent results. The majority of observational studies (Hogervorst et al 2000, Maki et al 2001, Yonker et al 2006), as well as randomized clinical trials in younger postmenopausal women (< 65 years old) have suggested beneficial effects of estrogen (Sherwin 1988, Shaywitz et al 2003, Joffe et al 2006) whereas long-term randomized clinical trials have failed to demonstrate improvements in cognitive function and some have even shown harmful effects (Rapp et al 2003, Espeland et al 2004, Resnick et al 2006, Resnick et al 2009, Yaffe et al 2006). It has been suggested that the diverse effects could be due to different age at initiation of treatment, time since menopause and type of regimen i.e. estrogen only or combined estrogen progestin therapy (Sherwin and Henry 2008, Maki and Sundermann 2009).

Data on cardiovascular disease from the large randomized Women's Health Initiative trial suggest that there may be an age dependent "window of opportunity" for the estrogenic effect (Russouw et al 2002). For women after the age of 60 years, estrogen treatment appears to give no benefit and rather to be associated with somewhat adverse effects. In contrast, for younger postmenopausal women estrogen treatment may offer cardioprotection. It could be that such an age dependent "window of opportunity" also exists for the potential effects of sex hormones on the brain. Data from the Study of Women's Health Across the Nation suggested that menopause transition-related cognitive difficulties may be time limited (Greendale et al 2009). While perimenopause was associated with impairment in cognitive performance, women with a stable postmenopausal status had an improvement and did not differ from younger premenopausal women.

The women in the present trial (*II*) were 7-8 years postmenopause with a mean age of 58-59 years, and were thus not in the age regarded as "window of opportunity". We found no significant influence by age, time since menopause or prior use of HRT. Most of the women were highly educated and it could be argued that this population was

strongly selected and not likely to benefit from the treatment. Considering that the mean result of the verbal memory test was ~ 25 out of maximum 48 scores and the mean result of spatial ability was ~ 9 out of 40 scores, “ceiling effects” appear not to be the case in our study. The duration of therapy may also be of importance. Our study was a short-term investigation and it cannot be excluded that longer treatment could have resulted in other outcomes.

Still in the present work (*III*), there were several significant correlations between serum hormone levels and some aspects of cognitive function, in particular for spatial ability. High absolute estradiol levels, a high estradiol/testosterone ratio and increasing levels during estrogen treatment were all associated with lower spatial ability. This is in agreement with many previous reports of sex differences, where men in general tend to outperform women in this specific aspect of cognitive function (Kimura and Clarke 2002).

In menstruating women, a deterioration of spatial ability was demonstrated in midluteal phase of the menstrual cycle when circulating levels of estradiol are high and an improvement during the follicular phase when estradiol levels are low (Hampson 1990, Maki and Rosenbaum 2002). In paper *III*, we found a similar association between estradiol levels and spatial ability in postmenopausal women after one month of estrogen treatment. There was also a negative association between spatial ability and oxytocin levels. This finding is probably a reflection of the estrogen-induced increase in oxytocin (Shukovski et al 1989). However, no significant association between sex hormones and spatial ability was found in the placebo and testosterone groups.

The influence of testosterone and its metabolites on spatial ability in women is complex and poorly understood. Some studies have shown positive associations between visuospatial performance and endogenous testosterone in women (Hausmann et al 2000, Hogervorst et al 2004). Furthermore, a single supraphysiological dose of testosterone was reported to improve visuospatial memory in young women (Aleman et al 2004). Still, other researchers have failed to find any significant positive relationship between testosterone levels and visuospatial abilities (Mofat et al 1996).

In our study (*III*), we found no direct correlation between endogenous testosterone and spatial ability. However, levels of the androgen metabolite ADG were positively

associated with spatial performance in the estrogen treated group, which to our knowledge is a new finding. Interestingly, this association was even further enhanced in a subgroup of women with low estradiol levels. The present finding is in line with the suggestion that the ratio of estradiol/testosterone is more important than the absolute serum level of testosterone for the effect on cognitive functions in women (Sherwin 2009). Maybe an association between ADG and visuospatial ability appears only in an environment with optimal estrogen levels. Local testosterone aromatization within the brain can rapidly control the local estradiol concentration (Hogervorst et al 2009).

Previous research, furthermore suggests that an optimal androgen level may exist and that there could be a curvilinear relationship between sex hormone levels and visuospatial ability (Moffat and Hampson 1996, Muller et al 2005). Recently, also evidence for a non-linear association between economic preferences and levels of endogenous testosterone was demonstrated (Stanton et al 2012). A tentative curvilinear relationship might also contribute to the lack of positive correlations in the testosterone treated group.

In the present study (*III*), we also found correlations between sex hormones and verbal fluency and verbal memory. There was a negative relationship between testosterone, as well as estradiol/testosterone ratio, and verbal fluency in the placebo group. This is in agreement with a population-based study where free testosterone was negatively associated with verbal fluency, semantic and episodic memory in women (Thilers et al 2006). However, some studies have failed to demonstrate a correlation between testosterone and verbal fluency (Wolf and Kirschbaum 2002).

In the estrogen treated group, there was a positive association between ADG and verbal memory in women with high estradiol levels but not in those with low estradiol levels. This finding may reflect the importance of the estradiol/androgen ratio for cognitive function, as well as the possibility that androgens may act indirectly by aromatization to estrogen. A similar finding was reported in older women, where both endogenous estradiol and testosterone levels were positively correlated to verbal memory (Wolf and Kirschbaum 2002).

Our results may be taken to support the hypothesis that a well-balanced relationship between estrogen and testosterone is crucial for cognitive abilities. The treatment

effects on the brain may be expressed as a result of both hormones mutually dependent on each other and their metabolites. Furthermore, a curvilinear relationship between sex hormone levels and cognition appears to exist.

### 5.3 SEX HORMONE TREATMENT EFFECTS ON THE SEROTONIN TRANSPORTER

To our knowledge this is the first study to demonstrate *in vivo* changes of 5-HTT BP after estrogen and testosterone administration in postmenopausal women.

Menopause represents a time of vulnerability for onset of depressive disorders (Rubinow 1998, Soares 2008). It has been shown that postmenopausal women on estrogen show greater improvement in mood during SSRI treatment than women without estrogen (Schneider et al 2001, Rasgon et al 2007). So far, there are only few human studies on the effect of gonadal hormones on the serotonin system. An increase of serotonin 2A receptors in the prefrontal cortex following estrogen administration alone or in combination with progesterone has been demonstrated in postmenopausal women (Kugaya et al 2003, Moses et al 2000).

In the present study, we found significant reductions in 5-HTT binding after estrogen alone and after combined estrogen and testosterone treatment relative to baseline condition. The reduction in 5-HTT BP is thought to represent less serotonin reuptake sites which could act to increase the serotonin levels in the synapse. The finding suggest that estrogen may have a modulatory effect on the serotonin system acting similarly to SSRI on 5-HTT to improve depressed mood associated with hypoestrogenic states in women. Some clinical studies have reported positive effects on mood by HRT itself in menopausal women (Zweifel and O'Brien 1997, Soares et al 2001, Huttner and Shepherd 2003, Frey et al 2008). Also in the present very small material there were apparent effects by hormone therapy on mood and some aspects of cognitive abilities, such as verbal fluency.

Estrogen has been found to have neuroprotective effects in the aging brain and support for these findings comes from epidemiological studies in humans, as well as experimental models of brain function *in vivo* and *in vitro* (Norbury et al 2003). A

functional MRI study in postmenopausal women showed that estrogen therapy significantly increased activation in the inferior parietal lobule and right superior frontal gyrus during verbal encoding and decreased activation during nonverbal coding (Shaywitz et al 1999). The findings suggested neuroprotective effects of estrogen on language related brain areas. Furthermore, estradiol and progesterone were found to upregulate cortical activity in brain regions (prefrontal, parietal and temporal cortices and hippocampus) that are important in the regulation of mood (Berman et al 1997).

Postmenopausal women are at risk for androgen deficiency. In surgically menopausal women the addition of testosterone to estrogen replacement therapy was found to improve psychological general well-being (Shifren et al 2000, Nathorst-Böös et al 2006). Animal data suggest that testosterone could have effects on the serotonergic system by acting via estrogen receptors (Bethea et al 2002) and by local conversion to estrogen within the brain (Celotti et al 1997, Simerly 2002). So far, very few PET studies have assessed effects of testosterone on the human serotonin system. Positive associations have been found between testosterone and activation in brain regions of importance for mood and cognitive abilities (vanWingen et al 2009, Witte et al 2009, Bos et al 2010, Hermans et al 2010, Schöning et al 2010). In the present study, we found that addition of testosterone to estrogen therapy significantly enhanced letter and category fluency and reduced 5-HTT binding relative to baseline in the same cortical areas as by estrogen treatment alone, as well as in several limbic areas. The mechanism behind this is not clear but possibly the effects are mediated via conversion of testosterone to estrogen. In support of this assumption, the mean level of estradiol was further enhanced after combined estrogen and testosterone treatment than by estrogen alone. Although our preliminary data provide novel evidence for effects of estrogen and testosterone on 5-HTT binding further research is required.

#### **5.4 A CRITICAL EVALUATION AND FUTURE PROSPECTS**

In the present thesis, a large placebo controlled randomized trial (*I, II*) found no evidence for any effect of short term treatment with neither estradiol nor testosterone on economic behavior and cognitive function in healthy postmenopausal women. In contrast, a PET study (*IV*) on a very limited material demonstrated not only significant effects on 5-HTT BP in different brain regions but also a significant effect on mood and

cognitive abilities like letter and category fluency after hormone therapy. In addition, the randomized trial showed several associations between circulating sex hormone levels and visuospatial ability (*III*). The interpretation of these apparent discrepant findings is complex and not fully clear.

The randomized trial was short-term and resulted in relatively low estradiol and testosterone serum levels, which may have precluded a significant effect between treatment groups. Even though values for mean estradiol increased by a factor 7.9 from treatment, the values around 200 pmol/L only correspond to the early follicular phase in women of fertile age. In a few previous studies on young women where a single dose of testosterone showed an effect on economic risk behavior (van Honk et al 2004) and impairment in cognitive function (van Honk et al 2011), blood levels of testosterone were increased 10-fold as compared to 4-fold in the present study. Still, the levels of estradiol in our postmenopausal women treated with estrogen were similar to those obtained during HRT, which have been reported to alleviate hot flushes, sweating and other menopausal symptoms (Santen et al 2010). Also, the resultant hormone levels in the PET study were quite similar and for testosterone even somewhat lower, but on the other hand the treatment was maintained for a total of six months.

Even if a larger sample might theoretically have changed the results of the randomized trial, the number of two hundred participants is very large compared to most of the other studies in this field. Reports on an effect of oxytocin on trust were based on a sample size of 29 in each of two randomization groups (Kosfeld et al 2005). The present sample size in each of the three randomization groups is more than twice as large, which should give a reasonable statistical power to detect differences in economic behavior. Furthermore, the randomized trial was estimated to have considerable statistical power to detect significant differences in cognitive variables.

One tentative explanation of the discrepancy between the randomized trial and the PET study could be the difference in design. To avoid the influence of repeated testing in the randomized trial, no evaluations were performed at baseline, and therefore it was not possible to analyze potential within group effects of hormonal treatment. In the PET study, repeated testing was performed and significant intra-individual effects were obtained. It could be that a very large inter-individual variation in economic behavior



and cognitive abilities of the women in the randomized trial hampered the detection of significant results between treatment groups.

## 6 GENERAL CONCLUSIONS

The results of the present work have shown that:

- Four weeks of treatment with either testosterone undecanoate 40 mg/day or estradiol valerate 2 mg/day had no significant influence on economic behavior in postmenopausal women. This finding was based on a large number of women and several validated economic experiments were used. Therefore the results are unlikely to be spurious. However, the negative findings do not rule out the possibility that endogenous exposure to sex hormones could affect economic behavior. Also higher hormone doses and a longer treatment period could theoretically have changed the results.
- Also this short-term low dose hormonal treatment had no apparent effects on some cognitive abilities in postmenopausal women. Effects on verbal fluency, verbal memory and spatial ability after treatment with either testosterone or estrogen were no different from those of placebo. Validated cognitive tests were used and sessions were conducted by the same investigator. However, to avoid any influence of repeated testing in the trial no evaluations were performed at baseline, and therefore it was not possible to analyze potential within group effects.
- There is a general lack of knowledge about which testosterone levels are needed to elicit a response within the brain and other organs in women. Although, no effects from testosterone and estrogen treatment were found in a randomized clinical trial, there were still significant correlations between sex hormone levels and some aspects of cognitive function in postmenopausal women. High estradiol levels, a high estradiol/testosterone ratio and increasing levels of estradiol during estrogen treatment were all associated with lower spatial ability. It could be that a specific balance between estrogen and testosterone is required for optimal effects on spatial- and verbal abilities. There are also data to suggest that the estrogen/testosterone ratio and also a curvilinear relationship are more important than absolute hormone levels.

- Treatment with estrogen alone (transdermal estradiol 100µg/day) for three months and in combination with testosterone undecanoate 40 mg/day for further three months has significant influence on the serotonin transporter binding potential (5HTT BP) in different brain regions of postmenopausal women. This effect was demonstrated by positron emission tomography (PET) using the special ligand [11C]MADAM. These results corroborate previous findings that suggest an effect of estrogen on the serotonin system. Furthermore, estrogen alone and estrogen/testosterone treatment significantly enhanced mood and cognitive abilities like letter and category fluency in surgically postmenopausal women. Although based on a small sample of women these data provide novel evidence for the influence of sex steroid hormones on the serotonergic system. Clearly further research in this field is required.

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