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Biological Basis of Sex Differences in Psychopharmacology

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Editors

Biological Basis of Sex Differences in Psychopharmacology

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Preface

This volume attempts to answer the question of how sex influences brain and behaviour. It brings together experts in this field, psychiatrists and other mental health care professionals with preclinical researchers to review the latest work in this area and give a thorough overview of how males and females are different in terms of brain function and behaviour. This is followed by a clinical perspective, applying brain biology to explain why some illnesses are gender specific and how gonadal steroids are involved in the aetiology and symptomatology of psychiatric diseases and may be modulated to provide new therapeutic approaches to mental illnesses. Appreciation and improved understanding of sex differences will certainly lead to improvements in the diagnosis, tailored treatment approaches and hence outcomes for people suffering with mental disorders. This volume will be essential reading for all health care professionals.

Animal behavioural models of efficacy studies in pharmacology generally use male rodents. However, the presentation of sex differences in behaviour underscores the need to include female animals in basic research studies. Similarly, gender differences in the presentation, treatment and outcomes of mental illnesses are often overlooked. The biological basis of sex differences and its application in clinical disorders are important issues that are highlighted by the research discussed in this volume.

The volume is divided into two sections, the first deals with the importance of recognising and studying sex differences in brain function and behaviour in animal models. The second section is dedicated to the consideration of biological sex differences in the presentation of aspects of mental illnesses such as schizophrenia, bipolar disorder, depression and anorexia nervosa. The two sections are interrelated and provide an integrated approach with animal research informing the human application in considering the biological basis of sex differences in psychopharmacology.

The volume starts with an overview by Professor Kay Marshall (a reproductive endocrinologist who has been persuaded to study the brain!) This chapter gives an important introduction into the mechanisms by which gonadal steroids produce their effects which explains how sex differences come about. The second chapter by Berend Olivier and colleagues explores how sex matters for rats. They provide an

overview of rodent sexual behaviour and how to measure this in the laboratory. The chapter has a particular emphasis on the role of serotonin (5-HT) on sexual activity and on sexual dimorphism in response to serotonergic agents. This is clearly of much relevance as drugs such as SSRIs cause sexual dysfunction in the clinic, and it is essential to model this appropriately in the laboratory. In a later clinical chapter on the impact of sex on antidepressants, John Sramek and colleagues detail clinical trial work on this important area. An investigation into sex differences and the effect of gonadal steroids on cognitive function in rodents is provided by Jane Sutcliffe, which is of particular importance as cognitive dysfunction occurs in many psychiatric illnesses such as depression, ADHD and PTSD. In schizophrenia most notably this remains an unmet clinical need, with emphasis on the development of new therapies for cognitive and other symptoms of this illness. Implications for the aetiology of schizophrenia are explored by Veena Kumari in her chapter dealing with human sensorimotor gating. Chapter 9 written by Anita Riecher-Rossler and Jayashri Kulkarni, and Chapter 10 by Angelika Wieck present the very important role that oestrogen plays as a key neuroprotective agent, and its impact on the timing and gender differences in illness presentation. The possibility of using hormone modulation as a new treatment approach is also discussed with respect to psychotic disorders.

Chapter 4 by Dai Mitsushima illustrates that in rodents neurotransmitters show sexual dimorphism, and that neurotransmitter release is affected by gonadectomy with a focus on acetylcholine, again of particular importance for cognition. A subsequent chapter by Justin Anker and Marilyn Carroll deals with the very important topic of drug dependence. They show that females are more sensitive than males to the reinforcing effects, and less sensitive to withdrawal effects, of certain drugs of abuse, making them more vulnerable to drug dependence which can be effectively modelled in animals. The translation from animals to humans here is impressive with female rats showing greater propensity for drug self-administration and relapse in animal models. The authors go on to demonstrate how these effects in females may be mediated by gonadal steroid hormones, in particular oestrogen and progesterone (which is important, as it is not all about oestrogen, as Kay Marshall explains in her opening chapter). They discuss possible mechanisms including oestrogen receptors and their interaction with the mesolimbic dopamine system with the emphasis on addiction to psychostimulants such as cocaine. Applying this framework, a novel proposal for the noted sex differences in anorexia nervosa is described in a chapter by Charlotte Keating, with applicability for new thinking about the aetiology of this severe and female dominant eating disorder.

Stress is of course an important feature of human lives, including our response to drugs of abuse and Christina Dalla and her colleagues cover this topic in some depth in their chapter. Men and women differ in their vulnerability to stress and stress-related psychiatric disorders such as depression. The authors explore sex differences in the response to acute and chronic stress in several animal models in some detail. Male and female rodents differ in their reactivity and adaptation to various stressors, and the authors demonstrate the link between this and differences in the

neuroendocrine system and its interaction with neurotransmitter systems, such as serotonin and dopamine. The final preclinical chapter provides an elegant review by Elizabeth Tunbridge and Paul Harrison into sex differences in the catechol-*O*-methyltransferase (COMT) gene. The gene encodes an enzyme that metabolises catechol compounds including dopamine, and the authors explain how sexual dimorphism in this gene and its interaction with oestrogen impacts on psychiatric disease states. Many of the preclinical studies suggest that sex-specific interventions may be a beneficial approach when treating patients, and understanding the sex differences in developmental disorders experienced early in life is an important area detailed by Bruce Tonge and colleagues in a clinical chapter that also proposes treatment strategies for early psychiatric presentations.

In summary, sex differences are observed in humans and animals in brain function and behaviour and in the response to illness. Men and women have different advantages in many aspects of behaviour particularly cognitive function which is a key component of many psychiatric disorders. Indeed, there is both clinical and preclinical evidence to support a role for the sex steroids in modulating performance in certain cognitive domains. At present, these interactions are complex and the underlying mechanisms have yet to be elucidated. Once these relationships are understood, there is potential for more effective therapeutic exploitation. This volume covers in some depth many illnesses in which sex differences and gonadal steroids are important in terms of aetiology, symptomatology, progression and treatment. It is the first volume to successfully achieve this and will be of considerable importance to workers in all aspects of mental illness.

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Introduction to the Interaction Between Gonadal Steroids and the Central Nervous System

Kay M. Marshall

Contents

Abstract The sex steroids are frequently referred to as the gonadal steroids and are erroneously assumed to be exclusively linked to the ovaries in women or the testes in men and the functions of the reproductive tract. This chapter will provide an overview of some of the extragonadal effects of these hormones, focusing on the central nervous system, and the mechanisms of hormone action. Hormone synthesis and metabolism within the CNS will be discussed with particular focus on the role of aromatase. Sex steroids exert many of their effects via intracellular receptors and these genomic responses tend to be slow in onset, however, some responses to steroids occur more quickly and are mediated via membrane receptors and involve interactions with many different transduction pathways to produce a diverse array of responses. These complexities do pose challenges but also offer opportunity for novel approaches for therapeutic exploitation as the pharmacological tools with which to modulate systems become increasingly available.

Keywords Androgen · Mechanism of action · Oestrogen · Progesterone

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The sex steroids are commonly referred to as the gonadal steroids and are erroneously assumed to be exclusively linked to the ovaries in women or to the testes in men and to the functions of the reproductive tract (Chabbert Buffet et al. 1998; Genazzani et al. 2002; Mooradian et al. 1987). The sex steroids themselves are not gender specific; for example, testosterone has several key functions in women; testosterone imbalance has been linked to depression in women (Rohr 2002) and oestrogen promotes hippocampal neurogenesis in male rats (Bowers et al. 2010). These steroids can be synthesised at extragonadal sites and have effects that are outside those obviously linked with reproductive processes. This chapter will provide an overview of some of the extragonadal effects of these hormones, focusing on the central nervous system (CNS), and the mechanisms of hormone action. The examples cited will be somewhat oestrogenocentric as this reflects the perhaps disproportionate amount of research time spent on oestrogen (a simple Medline search for 2010 suggests that twice as many papers were published on oestrogen than on testosterone). However, progesterone and testosterone and their related steroidal products should not be overlooked as it is often the balance of these hormones and/or their metabolites with one another that will determine the overall response. For example, progesterone can reverse the effects of oestrogen on dendritic spine density and on brain-derived neurotrophic factor (Murphy and Segal 2000; Aguirre et al. 2010). In addition, all responses mediated by sex steroids will in turn be influenced by chronobiology beginning in utero (Pilgrim and Hutchison 1994) and including the lifting of the hypothalamic–pituitary block that facilitates the onset of puberty. The reactivation of the hypothalamic gonadotrophin-releasing hormone (GnRH) secretory system (which can be stimulated by noradrenaline and glutamate and inhibited by GABA) results in the establishment of reproductive rhythms. In women, these can be disrupted by pregnancy, where the magnitude of the hormonal changes far exceed those occurring during the menstrual cycle (for detailed review, see Brunton and Russell 2010), and end at menopause or in the male, the increasingly recognised, although less clearly demarked andropause (Keenan and Veldhuis 2009). The timing of these biological life events has considerable impact on, and importance for, long-term health.

1 Hormone Levels in the CNS and Role of Aromatase

For a recent review of steroid hormone biosynthesis, see Gilep et al. (2011). The extent to which peripheral levels of either endogenous or exogenous circulating hormones reflect levels in the CNS is still subject to scrutiny. It is known that the sex steroids can be synthesised in the brain; for example, progesterone is synthesised by glial cells (Garcia-Segura and Melcangi 2006). A recent study by Caruso et al. (2010) measured levels of sex steroids in plasma and in the CNS (cerebellum, cerebral cortex and spinal cord) in intact and gonadectomised male and female rats and found that after gonadectomy, changes in the CNS did not necessarily reflect the situation in the plasma. In addition to this, there is the further complication of identifying which hormone is the active moiety. For example, there are several forms of endogenous oestrogen namely: 17β-estradiol (E2), which is the most

active and has the highest receptor affinity; estrone (E1), which is a less active product of oxidation of E2; estriol (E3), which can be produced from either of the former or from the androgen androstenedione. E3 is produced in abundance during pregnancy [it has been postulated that this happens to protect the developing CNS in the foetus (Reyes-Romero 2001)] but after the menopause levels do not really change and are similar to those found in men. 17α-estradiol, another form of endogenous oestrogen, has lower receptor affinity, but it is known to be synthesised locally in rodent brain (Simpkins et al. 1997; Levin-Allerhand et al. 2002). However, Nguyen et al. (2011) have recently reported measuring all of these oestrogens after derivitisation (using liquid chromatography separation, electrospray ionisation and tandem mass spectrometry, ESI-MS/MS) in human cerebrospinal fluid taken from trauma patients and E2 was found to predominate. This may represent assay or sample limitation; alternatively it could reflect another species difference and steroidogenesis does differ between species (Gilep et al. 2011). The synthetic pathways and resultant products (including metabolites, some of which retain biological activity) are determined by the types of steroidogenic cytochromeP450s (CYPs) and the dehydrogenase enzymes. In addition to inter-species variation, there is also some intra-species variation as the CYPs are subject to polymorphisms which may influence hormone as well as drug metabolism. Hormone metabolism may also explain some of the apparently paradoxical responses observed; for example, when the endogenous metabolite 2-methoxyestradiol is formed, it may possess some of the antioxidant properties of E2 but unlike E2 it cannot protect hippocampal neurones from insult by kainic acid (Picazo et al. 2003). Furthermore, E2 metabolism can lead to redox cycling and free radical formation and neural damage can ensue (Liehr and Roy 1990; Picazo et al. 2003).

In terms of variation between peripheral versus central steroid levels, dehydroepianstrosterone (DHEA) is a good example as DHEA can be detected in brain tissue of many species, including humans, at levels that exceed those in the periphery (Baulieu and Robel 1996; Tunbridge and Harrison 2011). DHEA is a substrate common to both oestrogen and androgen biosynthesis, and it appears that astrocytes from different brain areas can metabolise DHEA differentially; for example, hypothalamic cells are more active in producing E2 than similar cells from the cerebral cortex (Zwain and Yen 1999). However, there are some metabolic consistencies, as characterisation of the 5α-reductase-3α-hydroxysteroid dehydrogenase complex (which is key to androgen metabolism) in human brain samples has indicated no sex-specific differences and no differences over time (Steckelbroeck et al. 2001), which may suggest a non-reproductive role such as catabolism of neurotoxic steroids. Oestrogens can also be metabolised in the brain as indicated by high levels of 2- and 4-hydroxyoestrogens (the catecholoestrogens) which are metabolised further by catecholeamine-*O*-methyltransferases, an enzyme which itself is subject to oestrogenic influence and polymorphisms (Harrison and Tunbridge 2008).

The role of cytochrome P450 aromatase which is the protein product of gene *Cyp 19* also needs to be taken into account when considering the level of steroid hormones in the brain. This enzyme is responsible for converting androgens (C19 products) to oestrogens (C18 products), namely testosterone to E2 and

androstenedione to E1; thus it plays a key role in regulating the androgen–oestrogen balance. Aromatase is present at extragonadal sites including the breast (where its role and inhibition, using aromatase inhibitors or AIs, have been most extensively studied; for more details, see Furr 2006) and the brain where it appears to be concentrated in the preoptic area, ventro-medial hypothalamus and the bed nucleus striae terminalis (Balthazart et al. 2003). In terms of clinical relevance, an example would be the expression of aromatase by astrocytes following injury (Garcia-Segura et al. 2003). Aromatase is subject to different regulatory controls; for example, in the gonads cAMP is stimulatory but in neural tissues it is inhibitory (Lephart 1996). Interestingly in rat brain, androgen is not only the substrate for aromatase, but dihydrotestosterone can, like oestradiol, also induce its activity (Roselli and Resko 1993). This regulation can be slow (as would be expected if control is via genomic mechanisms) or fast (suggesting non-genomic mechanisms). The presence of phosphorylation consensus sites on the enzyme would correlate with the latter mechanism (Balthazart and Ball 2006). Neurotransmitters such as dopamine and glutamate can inhibit the activity of aromatase as can kainate and NMDA (Balthazart et al. 2003).

Other considerations when attempting to correlate levels of sex steroids with function could arise from the fact that only free, that is hormone unbound to plasma proteins such as albumin or more specifically in the case of the sex hormones, sex hormone-binding globulin (SHBG), hormone is active (the presence or absence of this globulin should also be considered when using animal models and extrapolating to the human). However, SHBG seems to have biological activity via a membrane receptor coupled to a cAMP pathway (Nakhla et al. 2009), and it may even be a marker for the integrity of the blood–brain barrier (Gustafson et al. 2007).

2 Mechanisms of Action

Sex steroids can act as ligand-activated transcription factors when they exert their effects via a genomic mechanism, and the response is slow in onset to allow for the eventual translation of message (see Fig. 1).

2.1 Oestrogen

In the case of oestrogen, these receptors are known as ERα and ERβ (Green et al. 1986; Kuiper et al. 1996), and for progesterone there are also two forms namely PR-A and PR-B. These receptors are part of the nuclear/steroid receptor superfamily (see Mangelsdorf et al. 1995). Oestrogen is also acknowledged to exert some of its actions via membrane receptors as discussed below.

Responses mediated by these receptors tend to be slow in onset to allow for subsequent modulation of gene transcription (Tsai and O'Malley 1994). ERα and ERβ share considerable sequence homology at the DNA (96%) and ligand (56%) (Weiser et al. 2008) binding domains and, in vitro at least, ERα is a stronger

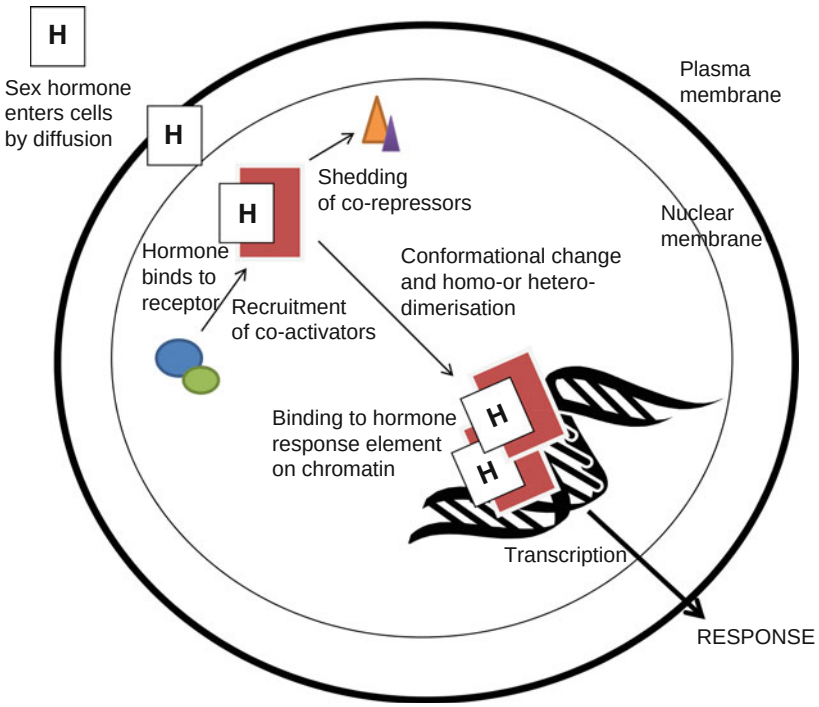

Fig. 1 Diagramatic representation of a sex hormone entering a target cell and binding to its receptor. The sex hormone (H) diffuses into the cell and binds to its intranuclear receptor, co-activator and co-repressor proteins are then recruited or shed (respectively) from the complex as it dimerises (this can either be with an identical complex, eg another ERα to form a homo-dimer or with a simlar complex eg an ERβ to form a hetero-dimer). This results in the remodelling or activation of the complex to facilitate its binding to the hormone response element of the chromatin which will in turn induce transcription and the response to the hormone (Beato and Sanchez-Pacheco 1996)

transcriptional activator (Delaunay et al. 2000). Effective transcription does require the recruitment of steroid receptor co-activators (SRCs) as shown in Fig. 1. These molecules influence several processes that follow ligand receptor interaction such as phosphorylation and thus modulate transcription (O'Malley 2006). They may also be involved in the aetiology of disease including neurological disorders (Lonard et al. 2007). The distribution of ERα and ERβ has been extensively studied in different species (see Österlund and Hurd 2001; Weiser et al. 2008).

ERα and ERβ differ not only in their distribution in the brain but also in their ligand-binding ability (Damdimopoulos et al. 2008) and roles (Bodo and Rissman 2006). A pertinent example could be oestrogenic protection against insult by NMDA in the hippocampus, an activity which is mediated by ERβ and induction of BDNF, a response inhibited by progesterone (Aguirre et al. 2010).

The potential for selective agonism or antagonism at receptor subtypes has been exploited therapeutically by, for example, the selective oestrogen receptor

modulators (SERMs) and the selective oestrogen receptor down-regulators (SERDs) such as tamoxifen and faslodex respectively, which have been used extensively in the management of breast cancer. Indeed, since the publication of the findings from the Women's Health Initiative (WHI) in the USA and in the UK, the Million Women Study (MWS) (for more information and resultant publications go to http://www.nhlbi.nih.gov/whi/references.htm and http://www.millionwomen-study.org/publications/ respectively), which in many respects were controversial not least with respect to the findings in relation to neuroprotection, the use of agents like the SERMs or even more selective compounds is likely to increase as hormone replacement regimens with better side-effect profiles are sought to manage the consequences of the menopause as women can now expect to live approximately a third of their lives in an oestrogen-deficient state. The central effects of the SERMS have yet to be fully evaluated, but they do have potential as tools and therapies (Arevalo et al. 2011) as summarised in Fig. 2.

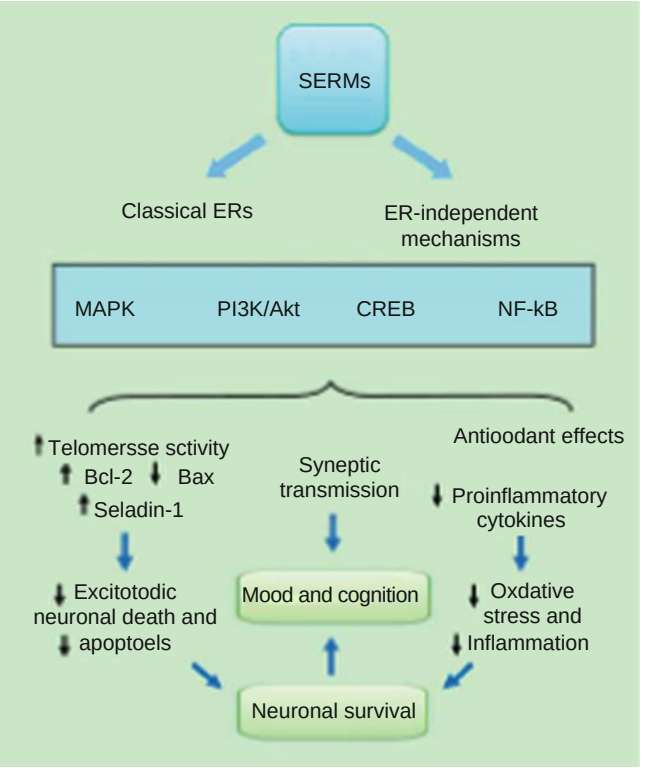

Fig. 2 Summary of the molecular mechanisms involved in the neuroprotective effects of SERMs. SERMs act in the nervous system through classical ERs or by ER-independent mechanisms and activate a variety of signalling molecules, including MAPK, PI3K, Akt, CREB and NF-κB. These molecules, in turn, trigger different coordinated mechanisms to promote neuronal survival and regulate mood and cognition (Journal of Molecular Endocrinology (2011) 46, 111–119)

In addition to the intracellular receptors, ERα and ERβ, there are also membrane receptors which are as yet less well defined. One such receptor is called GPR30 and is a 7 transmembrane domain G-protein-coupled structure (Lappano et al. 2010). Most of the work on this receptor has been done in relation to breast cancer (for review, see Maggiolini and Picard 2010), but GPR30 is expressed in the hypothalamic– pituitary axis, hippocampus and substantia nigra (Brailoiu et al. 2007). In the neo-cortex, a membrane-associated ER (ER-X) has been reported which mediates the activation of mitogen-activated protein kinase (MAPK), and 17αE2 appears to be the preferred ligand (Toran-Allerand 2004). The MAPK pathway can evoke the transcription of cAMP response element (CREB). 17βE2 can also interact with the phosphotidylinositol-3-kinase (P13K) pathway resulting in Akt (protein kinase B) activation which inhibits proapoptotic proteins.

E2 can also affect the monoaminergic systems as it can influence synthesis and degradation of dopamine, noradrenaline and 5-hydroxytryptamine as well as regulating monoamine reuptake transporters and second messengers. Findings from a study in female rats by Lubbers et al. (2010) indicate that SERMs may have the potential to manipulate monoamines selectively allowing some modulation of cognition and affective function. Oestrogens have several other central properties including (Amantea et al. 2005): an antioxidant effect principally due to direct free radical scavenging; anti-inflammatory action via suppression of interleukin-1β induction of COX-2 (for review and information on structure–activity relationships, see Prokal and Simpkins 2007). Oestrogen can also rapidly induce nitric oxide synthase activity in cortical neurones including in the hippocampus (Mannella et al. 2009).

2.2 Progesterone

The progesterone receptor also has two known subtypes namely PR-A and PR-B, the former being a truncated form of PR-B. PR-A dominantly represses the transcriptional activity mediated by PR-B (Giangrande and McDonnell 1999). These receptors are distributed in the human brain (Bezdickova et al. 2007) including in the hippocampus and frontal cortex (for review of form and function, see Brinton et al. 2008). Increased expression of PR-A can increase responsiveness to oestrogen via ERα (Mesiano 2001). Membrane receptors for progesterone, coupled to G-proteins, have also been identified that are associated with more rapid actions of the hormone (Thomas 2008; Dressing et al. 2011). Interestingly, unlike with PR-A and PR-B, many of the synthetic progestogens (see below) do not bind to the membrane receptor.

Progesterone, like oestrogen, has effects beyond those well recognised in the hypothalamus. Progesterone can also activate the MAPK and ERK signalling pathways which, can via CREB, lead to up-regulation of *bcl-2* in hippocampal neurones (Nilsen and Brinton 2002). Progesterone has been found to up-regulate BDNF in murine models of cerebral ischaemia (Coughlan et al. 2009) and may play a role in maintenance of the integrity of the blood–brain barrier (Ishrat et al. 2010).

A small clinical trial involving patients with traumatic brain injuries showed that the use of progesterone was not harmful and it may indeed have some beneficial effects (Wright et al. 2007). Phase III trials are now underway.

Selective agents for progesterone receptors have also been used experimentally to investigate further the role of progesterone. Therapeutic application of these agents will follow, and one agent, ulipristal acetate (ellaOne), was licenced in May 2009 in the UK for emergency contraception within 120 h (5 days) of unprotected intercourse or contraceptive failure. Ulipristal is an orally active synthetic selective progesterone receptor modulator (SPRM) with high affinity for PR-A. However, in animal studies it also has affinity for the glucocorticoid receptor, and in animals antiglucocorticoid effects have been seen. However, these antiglucocorticoid effects have not been seen in humans to-date and this may be indicative of real species differences.

A variety of synthetic progestogens are used in hormone replacement therapy and contraception, as progesterone itself has poor oral bioavailability. Not all progestogens have the same pharmacological profile (Hapgood et al. 2004), and these differences have implications for their usage. Two of the most widely used synthetic progestogens are medroxyprogesterone acetate and norethisterone. These are used as the progestogenic component of an HRT regimen in combination with oestrogen but have been shown to increase the risk of breast cancer in long-term HRT users (Million Women Study 2003; Women's Health Initiative 2002). Structurally, medroxyprogesterone acetate is more similar to natural progesterone than norethisterone. The metabolism of these two compounds is also different, as medroxyprogesterone acetate is the major progestogenic compound rather than its metabolites. In contrast, the metabolites of norethisterone (a first generation progestagen) exhibit significant activity in addition to a wide range of non-progestogenic actions. Norethisterone also binds to SHBG, whereas medroxyprogesterone acetate does not. The most notable difference in steroid receptor-binding affinity between the two synthetic progestogens and endogenous progesterone is that, although all the compounds have affinity for the mineralocorticoid receptor, only the natural compound has antagonist activity. MPA is the most potent of the three in terms of glucocorticoid activity and correspondingly it has the highest affinity for the glucocorticoid receptor, but this relationship is complicated further by the fact that the resultant effect appears to be dependent on glucocorticoid receptor density (Hapgood et al. 2004). Unlike endogenous progesterone, both MPA and the 19-nortestosterone derivatives also have affinity for the androgen receptor (but MPA has less intrinsic activity), although this is decreased in the third-generation compounds such as desogestrel. Such off-target pharmacology influences the side-effect profiles of these synthetic analogues.

In addition, natural progesterone and its metabolites allopregnanolone and pregnanolone have high affinity for $GABA_A$ receptors (Paul and Purdy 1992), and progesterone can decrease glutamic acid decarboxylase (GAD) and so attenuate GABA synthesis (Wallis and Luttge 1980). The GABAergic system can also be influenced by the androgen metabolite 5α-androstane-3α,17β-diol (3α-Adiol), which is a metabolite of the potent androgen dihydrotestosterone (DHT) which is formed from testosterone by the action of 5α-reductase, and can modulate $GABA_A$ receptors (Reddy 2004). 3α-Adiol is in turn metabolised by CYP7B1 which is

expressed in the hippocampus, and this metabolic route may, therefore, indirectly influence $GABA_A$ pathways (Pettersson et al. 2009).

2.3 Androgen

As with oestrogen and progesterone, it is recognised that androgens may also contribute to CNS physiology and pathology outside the control of reproduction. The two most important androgens are testosterone which is transformed within the CNS by 5α-reductase to 5α-dihydrotestosterone (DHT) (testosterone can also undergo aromatisation to estradiol as discussed earlier), and they mediate their effects, like oestrogen and progesterone, via a ligand-activated transcription factor, namely the androgen receptor (AR). Much of the knowledge of the AR has been derived from work done in the field of prostate cancer (Powell et al. 2004). In the CNS, mapping of AR distribution has revealed that there is a high level of co-localisation with ERs (Patchev et al. 2004). Overlap also occurs with respect to AR expression as in, for example, the male rat forebrain oestrogens up-regulate expression. Androgen withdrawal (via castration) can increase expression in some brain areas (preoptic nucleus) but have no effect in others (amygdala); these effects are also influenced by age (Kumar and Thakur 2004), and that perinatal or even prenatal androgen exposure may be important (Goren and Kruijver 2002). Therefore, there appears to be age-dependent, hormone and tissue-specific control of the AR. The influence of androgens outside reproduction is less clear than for oestrogen and progesterone; however, high levels of AR mRNA have been detected in human hippocampus (Beyenberg et al. 2000).

There is evidence to suggest that testosterone and DHT can, like oestrogen, influence neuronal plasticity (Matsumoto and Prins 2002) and dendritic spine density (Leranth et al. 2004). Androgens may also reduce neuronal cell death after exposure to various insults, such as β-amyloid (Pike 2001) and oxidative stress (Ahlbom et al. 2001). These effects could be mediated via AR activation of MAPK/ERK signalling pathway (Nguyen et al. 2005). Androgens may also be involved in the regulation of several proteins involved in axonal regeneration such as neuritin and tubulin, and there is evidence that androgen action is potentiated by BDNF (Fargo et al. 2009). The complexities of these mechanisms may allow for selective activation of signalling pathways, and selective androgen receptor modulators (SARMs) are being developed to specifically manipulate certain effects; as yet these are limited to the periphery to improve outcomes in the management of prostatic disease, osteoporosis and muscle wasting (Gao and Dalton 2007).

3 Conclusion

The effects of the sex steroids in the brain, begin in utero (Swaab 2007), are complex, and the final response is dependent not only on receptor up- or down-regulation but also on a series of coordinated metabolic events and cross-talk

between receptor signalling pathways. The importance of each step may vary depending on the surrounding milieu, the tissue, the species and the gender of the species. Gender differences are important and should not be discounted in the interest of sample homogeneity. Findings from research using males may not always be valid when extrapolated to cover the female population (Beery and Zucker 2011), and a case could be made for sex-specific medicines (Gillies and McArthur 2010). These complexities do pose challenges but also offer opportunity for novel approaches for therapeutic exploitation and the pharmacological tools with which to modulate systems are becoming available from SERMs to SERDs.

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Differences in Sexual Behaviour in Male and Female Rodents: Role of Serotonin

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Contents

Abstract Serotonin plays an important role in both male and female sexual behaviour. In general, reduction of 5-HT function facilitates, whereas enhancement inhibits sexual behaviour. Most fundamental research on the involvement of 5-HT in sex has been performed in rats. Selective serotonin reuptake inhibitors (SSRIs)

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have comparable effects on male and female sexual behaviour in rats; they inhibit it but only after chronic administration. Activation of the 5-HT_{1A} receptor facilitates sexual behaviour in male rats but inhibits sexual behaviour in female rats, suggesting a differential role for 5-HT_{1A} receptors in male and female rats. Research on sexual behaviour in rats with null mutations in the serotonin transporter (SERT) indicated also a differential role for 5-HT_{1A} receptors in male and female sexual behaviour. Evidence exists that different pools of 5-HT_{1A} receptors have differential roles in various parts of the cascade of sexual events occurring during sexual interactions. Roles for other 5-HT receptors are less well defined although 5-HT_{1B}, 5-$HT_{2A/B}$ and 5-HT_7 receptors seem to be involved. Identification of putative differential or comparable roles in female and male sexual activities requires more research.

Keywords 5-HT · 5-HT_{1A} receptor · 5-HTT · 8-OH-DPAT · Gender · Hypersexual behaviour · Hyposexual behaviour · Paroxetine · Premature ejaculation · Retarded ejaculation · Serotonin · Serotonin receptor knockout rat · Serotonin transporter · SERT polymorphism · Sexual behaviour · SSRI · WAY100635

1 Introduction

Sexual behaviour in rodents (and we strictly focus on the rat) happens when animals reach adulthood and engage in behaviours that result in the joining of a male and female, ending in copulation, with the intent to reproduce. The female rat's sexual behaviour is dependent on the reproductive cycle, whereas the male's sexual behaviour is not. The female's sexual behaviour is strongly dependent on peripheral gonadal steroids that have both peripheral and central nervous system (CNS) effects. Steroids act on the brain to induce sexual receptivity and all associated behaviours (proceptive, receptive and pacing behaviours). Quite some work has been performed to delineate the neural circuitry and neurochemistry of female behaviour, especially from lordosis, a behaviour that is evoked by external stimuli, normally, a male rat. Lordosis behaviour is only observed when the female is hormonally (or naturally) primed (oestradiol + progesterone) and the circuitry involves sensory, brain, spinal cord and motoric activation. In the CNS, the ventromedial nucleus of the hypothalamus (VMH), the preoptic area (POA), the midbrain central gray (MCG) and two areas in the spinal cord (cervical and lumbar) are the key structures. All structures contain oestrogen receptors that seem essential for the final integrative performance of full sexual behaviour. Many neurotransmitter systems in the CNS regulate or modulate (aspects of) sexual behaviour, including serotonin. There is strong evidence that the serotonergic modulation of sexual behaviours mainly occurs at the level of the VMH and POA.

In male rodents (rat), testosterone (T) acts during development to promote genital development and organization of the CNS neural circuitry. In adulthood, the neural circuitry along with the appropriate sensory and motoric systems controls the male's sexual motivation and performance. Male rat's sexual behaviour includes penile erection, sexual motivation and mating behaviour. All can be

studied while observing the mating behaviour of a male rat in direct interaction with a receptive female. In such an interaction, the male approaches the female, sniffs her and starts mounting (the female displays lordosis). The male displays a series of mounts and intromissions that end in ejaculation. After an ejaculation, the male displays for some time sexual quiescence followed by the next series of mounts and intromissions, leading again to ejaculation, and so on. In the male rat, the testicular secretion of T occurs throughout the year, although pulsatile patterning occurs over the day. Seasonal variations in behavioural responsiveness to T of male rats have not been found, making the male (and female) rat ideal experimental animals to study the neural mechanisms of, and neurotransmitter involvement in, sexual behaviour.

The neural systems involved in male sexual behaviour seem to involve many structures that are also involved in female sexual behaviour, although clear differences are also notable. The POA and the bed nucleus of the stria terminalis (BNST) are core structures via which T acts to activate male sexual behaviour. In particular, the POA seems an integrative structure in coordinating the actions of T on both motivational and consummatory aspects of male sexual behaviour. Several neurochemical systems, including peptidergic, dopaminergic and serotonergic systems, play a role in mediating sexual behaviour.

2 Serotonin and Sexual Behaviour

The focus of this chapter is the role of serotonin in male and female sexual behaviour in the rat. There is hardly any research performed on gender differences in the development and adult functioning of the 5-HT system in the brain and spinal cord. Seeing the overlap, but also the divergence of the various neural structures and hormonal receptor systems in the male and female rat CNS, it may be difficult to predict the effects of psychopharmacological treatment with serotonergic ligands on male and female sexual behaviour.

Serotonergic psychopharmacology in humans is rather limited; only the selective serotonin reuptake inhibitors (SSRI) are selective serotonergic drugs extensively used in patients, whereas most other drugs with some serotonergic profile exert inherently other mechanisms like dopamine D_2 receptor antagonism (olanzapine, risperidone, buspirone). In the latter case, it is often impossible to purely deduct the specific contribution of the serotonergic component on the putative effects on sexual behaviour or sexual dysfunctions induced. SSRIs are widely used to treat depression both in human males and females and are notoriously implicated (Zemishlany and Weizman 2008) in inducing sexual disturbances (Kennedy and Rizvi 2009; Balon 2006). However, a complicating factor is that major depression per se is often (if not always) associated with sexual disturbances (e.g. in libido, motivation, erection: Kendurkar and Kaur 2008; Kennedy and Rizvi 2009). SSRIs enhance serotonergic neurotransmission which is generally believed to inhibit sexual behaviour, both in males and females (Zemishlany and Weizman 2008; Williams et al. 2006; Kennedy and Rizvi 2009; Kendurkar and Kaur 2008). This is confirmed by various studies showing that SSRI antidepressants induce

sexual disturbances, in addition to already present dysfunctions due to the underlying depression, in both males and females (Cyranowski et al. 2004; Regitz-Zagrosek et al. 2008). No studies in humans have looked into the brain mechanisms underlying the SSRI-induced sexual dysfunction and putative gender differences. While it is still assumed that high extracellular 5-HT levels (e.g. after SSRI treatment) are needed to promote antidepressant activity, the disadvantage is the directly associated decrease in sexual behaviours. The emerging pattern seems to indicate that SSRIs, which enhance serotonergic neurotransmission in the brain, have similar inhibitory effects in human males and females. In line with the latter notion is the finding (Sugden et al. 2009) that gene expression for 5 serotonergic genes (including 5-HTT) did not differ between genders in postmortem human brains.

3 Serotonin, Serotonergic Receptors and Male Sexual Behaviour

3.1 Introduction

The importance of 5-HT in male sexual behaviour has been demonstrated by numerous studies showing that, for instance, lesions of the brainstem raphé nuclei (Albinsson et al. 1996) and 5-HT depletion (Tagliamonte et al. 1969) facilitate sexual behaviour. On the other hand, administration of 5-hydroxytryptophan, the direct precursor of 5-HT, 5-HT itself and 5-HT releasers such as MDMA and fenfluramine, inhibits sexual behaviour (Ahlenius et al. 1980; Dornan et al. 1991; Foreman et al. 1992; Gonzales et al. 1982). Altogether these findings suggest that a decrease in 5-HT neurotransmission may be involved in facilitation, whereas an increase in 5-HT neurotransmission may result in inhibition of male sexual behaviour.

3.2 SSRIs and Male Sexual Behaviour

The frequently reported sexual effects of SSRIs in men demonstrate an important role of 5-HT in human ejaculatory behaviour. In several human studies we and others have demonstrated that SSRIs including paroxetine, sertraline and fluoxetine are able to delay ejaculation in premature ejaculation (for review, see Waldinger 2002; De Jong et al. 2006). Moreover, these studies show that SSRIs exert only a minimal ejaculation delay in the first week that is often not clinically relevant. A clinically relevant ejaculation delay occurs gradually after 2–3 weeks of daily treatment. Interestingly, despite the putative similar underlying mechanism of action of SSRIs – briefly, preventing the reuptake of 5-HT, thereby elevating 5-HT levels – not all SSRIs delay ejaculation to the same extent. In humans, the tricyclic antidepressant, clomipramine and the SSRI, paroxetine have stronger ejaculation-delaying effects after 4–6 weeks of daily treatment than other SSRIs (Waldinger et al. 1998, 2001a, b).

3.3 Acute and Chronic SSRI Administration in Male Rats

Analogous to the human situation, in male rats a distinction can be made between the effects of acute and chronic SSRI administration on ejaculation. Acute administration of various SSRIs, such as citalopram, paroxetine, sertraline, fluoxetine and fluvoxamine, did not or marginally delay ejaculation (Mos et al. 1999; Ahlenius and Larsson 1999; Matuszcyk et al. 1998). On the other hand, chronic administration of fluoxetine (Matuszcyk et al. 1998; Cantor et al. 1999; Frank et al. 2000) and paroxetine (Waldinger et al. 2001a, b) delayed ejaculation in male rats. Nonetheless, as in humans, not all SSRIs potently delay ejaculation after chronic administration in male rats: fluvoxamine slightly affected some aspects of copulatory behaviour, but did not affect ejaculation (Waldinger et al. 2001a, b; De Jong et al. 2005a). It is unclear why the various SSRIs differ in their ability to delay ejaculation after chronic administration. The delay in onset of the therapeutic effect of SSRIs in depression and anxiety disorders has been related to adaptive changes of serotonergic autoreceptors (Haddjeri et al. 1998; Le Poul et al. 2000), and it is conceivable that the ejaculation-delaying effects of various SSRIs are due to differential adaptive changes of 5-HT receptors.

An example of the effects of an SSRI antidepressant (paroxetine) in male rat sexual behaviour is shown in Fig. 1. The effect is clearly seen in the number of ejaculations per 30-min test in sexually trained animals. Acutely (Day 1: 30 min after injection) paroxetine does not inhibit sexual behaviour whereas after 7 (subchronic; 5 and 10 mg/kg) or 14 days treatment (chronic; 2.5, 5.0 and 10.0 mg/kg)

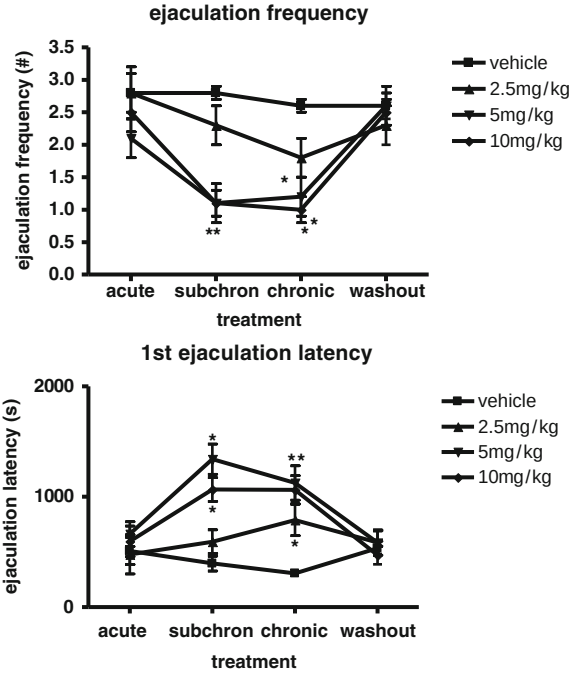

Fig. 1 The mean number of ejaculations ± SEM of male rat groups treated with vehicle or different doses of the SSRI paroxetine (2.5, 5.0 and 10.0 mg/kg IP) is given after acute (30 min pre-treatment), sub-chronic (7 days; once daily) and chronic (14 days: once daily) treatment. One week after cessation of treatment (washout), sexual behaviour was again measured but now without any treatment. Sexual behaviour tests were run on days 1, 7, 14 and 21 and consisted of a 30-min test in which a male rat had free access to a female that was hormonally brought into oestrus (method: Chan et al. 2010). *$p < 0.05$ compared to vehicle

paroxetine strongly (and dose dependently) reduces sexual behaviour. The effect is reversible as animals return to their pre-testing level 1 week after cessation of treatment. A similar picture emerges for the first ejaculation latency that is not affected acutely, but is dose-dependently enhanced after 7 days and 14 days of treatment, and returns to baseline 1 week after cessation of treatment.

Ahlenius and Larsson (1999) have studied the mechanism of SSRI-induced delay of ejaculation in more detail and showed that acute treatment with citalopram did not affect ejaculatory behaviour. Co-administration of the 5-HT_{1A} receptor antagonist WAY-100635 with citalopram strongly delayed ejaculation latencies, suggesting 5-HT_{1A} receptor involvement in the effect of citalopram on ejaculation. De Jong et al. (2005a, b) also showed that citalopram, acutely or chronically, while not inhibiting sexual behaviour itself, when combined with a sexually inactive dose of WAY100635 completely abolished sexual behaviour.

We studied this phenomenon further and confirmed earlier findings (Looney et al. 2005) that a dose as low as 0.01 mg/kg of WAY100635 facilitated the behaviourally inactive acute 10 mg/kg paroxetine dose and led to strong inhibition of male sexual behaviour (Fig. 2). The data suggest that the inhibitory action of SSRIs after (sub) chronic treatment are related to changes at certain 5-HT_{1A} receptors after long-term treatment.

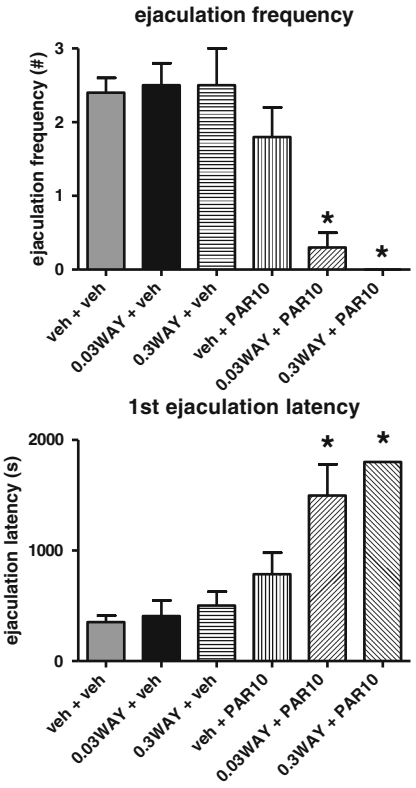

Fig. 2 Sexually trained male rats were acutely injected with saline or 10 mg/kg paroxetine (IP; 30 min before testing) immediately followed by an injection of either saline or a dose (0.03 and 0.3 mg/kg IP) of the 5-HT_{1A} receptor antagonist WAY100635. During an ensuing sexual behaviour test of 30 min, the sexual behaviour of the male was scored. In the figure, the mean number of ejaculations ± SEM is given. *PAR* paroxetine, *WAY* WAY100635, *VEH* vehicle. *$p < 0.05$ compared to vehicle

Subsequently, it was found that the ejaculation-delaying effects of the combination of citalopram and WAY100635 could be fully blocked by a selective 5-HT_{1B} receptor antagonist, suggesting a role for this receptor subtype in the delay of ejaculation (Hillegaart and Ahlenius 1998). Interestingly, a previous study from the same laboratory also suggested a role of the 5-HT_{1B} receptor in the delay of ejaculation. In this study, it was shown that the 5-HT_{1B} receptor agonist anpirtoline dose-dependently delayed ejaculation in rats (Hillegaart and Ahlenius 1998).

3.4 SERT-KO Rats and Male Sexual Behaviour

In humans, the SERT plays a prominent role in the homeostasis of serotonergic neurotransmission. Polymorphisms in the promoter region of the SERT influence the activity of SERT, and the two length alleles (S and L allele) have functional consequences for the function of the 5-HT system (Murphy and Lesch et al. 2008). L and S (LL > LS > SS) generate allele-dependent 5-HT activity with associated functional consequences (Lesch et al. 2008). Rats do not possess such promoter length polymorphisms but genetic knockout of the SERT gene might generate rat models of the S-allele versions of the human SERT. Therefore, $SERT^{-/-}$ and $SERT^{+/-}$ can be compared to wild-type ($SERT^{+/+}$) male rats and their sexual behaviour studied (Chan et al. 2011). It was expected, in analogy to treatment with chronic SSRI treatment, that $SERT^{-/-}$ and $SERT^{+/-}$ rats would display a lowered sexual behaviour compared to $SERT^{+/+}$ rats.

All rats (30 per genotype) were trained up to seven times (once weekly a test of 30 min) and gene knockout rats indeed showed lower sexual performance than wild-type rats. On average the mean number of ejaculations at week 7 was 1.6 for $SERT^{+/+}$, 1.1 for $SERT^{+/-}$ and 0.7 for $SERT^{-/-}$ rats (Fig. 3), a significant decrease

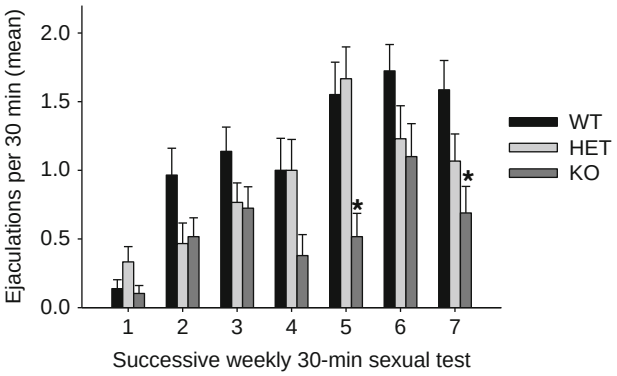

Fig. 3 Development of sexual behaviour (mean number of ejaculations/test) in male wild-type ($SERT^{+/+}$, WT), heterozygous ($SERT^{+/-}$, HET) and homozygous ($SERT^{-/-}$, KO) rats tested weekly over 7 weeks in a sexual behaviour test of 30 min with an oestrus female. *$p < 0.05$ compared to WT animals

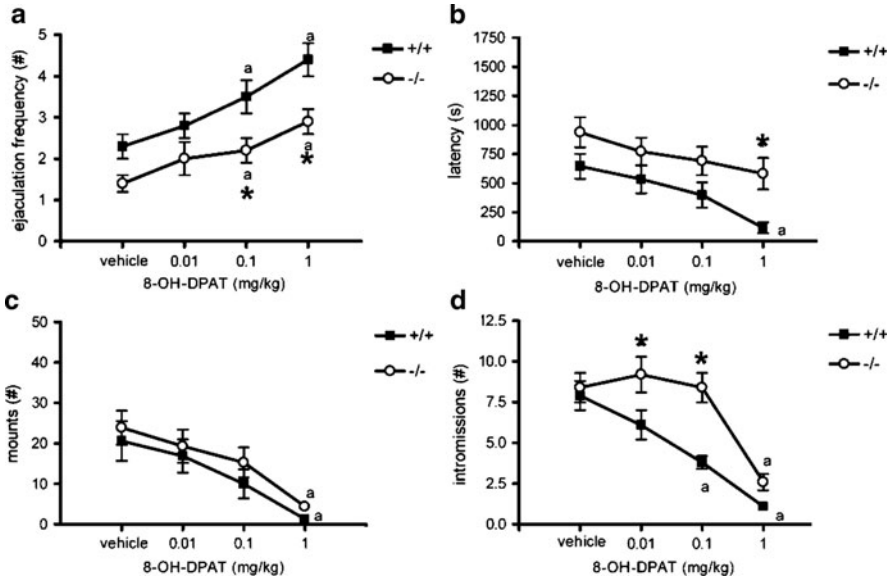

Fig. 4 Effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT (s.c.) on ejaculation frequency over a 30-min test (**a**), latency to first ejaculation (**b**), first ejaculatory series mounts (**c**) and first ejaculatory series intromissions (**d**) of $SERT^{+/+}$ (+/+) and $SERT^{-/-}$ (−/−) animals. *$p < 0.05$ compared to wild type (+/+); *a*: $p < 0.05$ compared to vehicle treatment

for the homozygote gene knockout. The heterozygote KO was not different from the wild type.

Next, the 5-$HT_{1A/7}$ receptor agonist +/−8-OH-DPAT was tested. 5-HT_{1A} stimulation has pro-sexual activities in rats which also occur in the three genotypes. Although the basal level of sexual behaviour (number of ejaculations, ejaculation latency, post-ejaculatory latency) in the $SERT^{-/-}$ is lower than in the other two genotypes (Fig. 4), the stimulant effect of 8-OH-DPAT in all three genotypes is similar, indicating that 5-HT_{1A} receptors mediating this effect have not changed [(de)sensitized].

The 5-HT_{1A} receptor antagonist WAY100635 had no effects in the WT and heterozygote rats but had a dose-dependent inhibitory effect in the SERT-KO (Fig. 5), suggesting that a different pool of 5-HT_{1A} receptors is involved in its action and that these receptors appear sensitized in the SERT-KO. Remarkably, the heterozygous $SERT^{+/-}$ rats did in no way differ from the WT rats. Heterozygous SERT-KO rats have intermediate enhanced extracellular 5-HT levels compared to WT and SERT-KO ($SERT^{-/-} > SERT^{+/-} > SERT^{+/+}$). Apparently, like the effective dose of SSRIs that need to occupy at least 80% of the SERTs before antidepressant efficacy is observed (Kugaya et al. 2003), the $SERT^{+/-}$ still has sufficient SERT capacity (50%) left to show undisturbed sexual behaviour.

To summarize, the sexual side effects of SSRIs are still not fully understood. Nevertheless, some recent findings and genetic evidence suggest that adaptive changes in the 5-HT system and probably its interactions with neuroendocrine systems (De Jong et al. 2007) may be responsible for their sexual effects.

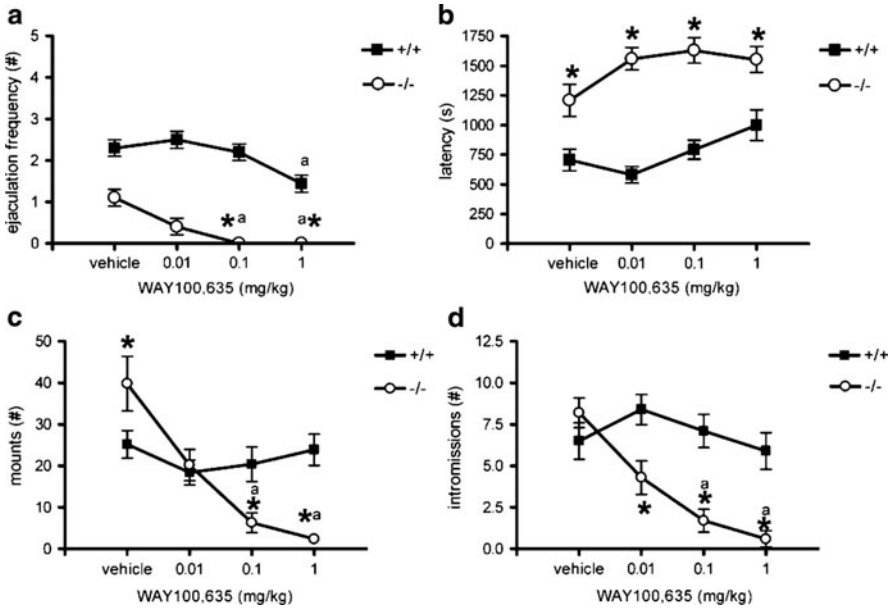

Fig. 5 Effects of WAY100635 (IP) on ejaculation frequency over 30-min test (**a**), latency to first ejaculation (**b**), first ejaculatory series mounts (**c**) and first ejaculatory series intromissions (**d**) of wild-type (+/+) and serotonin transporter knockout (−/−) animals. *$p < 0.05$ compared to wild type (+/+); *a*: $p < 0.05$ compared to vehicle treatment

3.5 Serotonin Receptor Agonists and Antagonists and Ejaculation in Male Rats

As described above, activation of 5-HT_{1B} receptors has been associated with delaying ejaculation in male rats. 5-HT_2 receptors are also implicated in modulation of sexual activity, e.g. shown by the 5-$HT_{2A/2C}$ receptor agonist DOI-induced inhibition of sexual behaviour (Klint and Larsson 1995). On the other hand, several other studies have shown that 5-$HT_{2A/2C}$ receptor agonists generally inhibit sexual behaviour by decreasing the number of animals that initiated copulation, but do not affect ejaculation latencies in animals that do initiate copulation (Ahlenius and Larsson 1998; Klint et al. 1992; Watson and Gorzalka 1991). Thus, it appears that 5-HT_2 receptors in general inhibit sexual behaviour, but their precise role in the regulation of ejaculation is not entirely clear.

A facilitatory role on ejaculation has been ascribed to activation of 5-HT_{1A} receptors, and various selective agonists for this receptor, such as 8-OH-DPAT (Ahlenius and Larsson 1990), FG-5893 (Andersson and Larsson 1994) and flesinoxan (Haensel and Slob 1997; Mos et al. 1991), potently facilitate sexual behaviour and decrease ejaculation latencies. Nevertheless, the underlying mechanisms of the facilitatory effects of 5-HT_{1A} receptor agonists are still unclear. A possibility for the mechanism of action may be activation of presynaptic 5-HT_{1A} receptors that

Table 1 Mean number of ejaculations, mounts and intromissions and ejaculation latency (in seconds) for sexually naïve male rats during a 15-min test with a sexually active, oestrus female

Drug (route)	Dose (mg/kg)	EF	MF	IF	EL (s)
8-OH-DPAT (SC)	0	0.1	10.5	8.3	869
	0.1	1.5[a]	6.5[a]	7.6	351[a]
	0.2	1.9[a]	3.5[a]	5.1	187[a]
	0.4	1.7[a]	1.1[a]	1.1[a]	238[a]
Flesinoxan (IP)	0	0.3	13.9	12.2	854
	0.1	1.0[a]	7.9	13.4	636[a]
	0.3	1.3[a]	5.5[a]	9.8	459[a]
	1.0	1.8[a]	3.3[a]	8.2	281[a]
Buspirone (IP)	0	0.3	10.3	9.9	860
	3.0	1.2[a]	7.0	11.3	502[a]
	10.0	0.1	0.3[a]	1.8[a]	849
Ipsapirone (IP)	3.0	0.9([a])	7.9	11.3	502[a]
	10.0	1.5[a]	10.9	12.2	636[a]

All data are depicted as means
EF ejaculation frequency, *MF* mount frequency, *IF* intromission frequency, *EL* ejaculation latency
[a]Significantly different ($p < 0.05$) from the corresponding vehicle (0 mg/kg) dose

will lead to an inhibition of 5-HT neuronal firing and consequently results in facilitation of sexual behaviour as described above. Alternatively, activation of postsynaptic 5-HT_{1A} receptors may result in facilitation of sexual behaviour. Evidence for a postsynaptic mechanism of action is provided by studies demonstrating that injection of 8-OH-DPAT directly into the medial preoptic area potently facilitated sexual behaviour and lowered ejaculatory threshold (Matuszewich et al. 1999). Administration of 5-HT_{1A} receptor antagonists does not lead to any change in sexual behaviour (Ahlenius and Larsson 1999; De Jong et al. 2005a; Sura et al. 2001). Moreover, the effects of 5-HT_{1A} receptor agonists can be antagonized by 5-HT_{1A} receptor antagonists. When 5-HT_{1A} receptor antagonists are combined with SSRIs (after acute or chronic administration), the inhibitory action of SSRIs is facilitated indicating a role for the 5-HT_{1A} receptor in the inhibitory action of SSRIs in male sexual behaviour (De Jong et al. 2005a, b; Table 1)

3.6 Animal Models of Premature and Retarded Ejaculation

Most of our current understanding of the anatomy and neurobiology of sexual behaviour is based on animal studies using rats that are sexually experienced and display normal sexual behaviour. Interestingly, the comparable ejaculation-delaying effects of SSRIs in humans and rats suggest high translational validity with regard to the regulation of ejaculation. Nevertheless, face validity is low when one tries to extend results obtained in rats that display normal sexual behaviour to dysfunction such as premature and retarded or even (an)-ejaculation. Over the last decades, several groups have studied rats that display hyposexual behaviour and are referred to, by different investigators, as sexually inactive, sluggish, impotent or

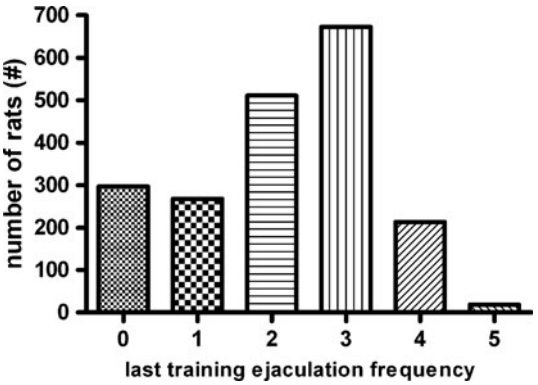

Fig. 6 More than 1,900 male rats were tested over a period of 5 years and trained weekly for 4 weeks in a sex test of 30 min against a female rat brought into behavioural oestrus. The graph represents the number of animals that displayed: 0, 1, 2, 3, 4 or 5 ejaculations during the last training test. Animals with 0 or 1 ejaculations/test were depicted as "slow" or "sluggish"; animals with two to three ejaculations/test as "normal" and animals with more than three ejaculations/test as "fast"

non-copulating rats. Recent findings suggest the presence of neurobiological differences associated with the hyposexual behaviour that these rats display. On the other hand, hypersexual behaviour can also be provoked pharmacologically. However, there are only few studies that have studied rats that are hypersexual by nature. Thus, investigating animals that do not display normal sexual behaviour may help understanding of the underlying neurobiological mechanisms and hopefully will provide further insight in the aetiology of ejaculatory dysfunction.

In our laboratory, we have found (Pattij et al. 2005; Olivier et al. 2005) that male outbred Wistar rats display sexual "endophenotypes". In subsequent cohorts of 100–120 male rats, we consistently found rats that display a very low (0–1), normal (2–3) or high (4–5) number of ejaculations in 30-min tests with a receptive female even after four to eight training tests. The behaviour of these males seems very stable, and we suggest the low performing animals as putative model for delayed ejaculation in humans and the high performing rats as model for premature ejaculation (Pattij et al. 2005; Olivier et al. 2006). Figure 6 shows the distribution of these "endophenotypic" sexual phenotypes in 1,982 male rats we tested thus far.

These various endophenotypes are now the subject of pharmacological studies.

3.7 Studies with Rats Displaying Hyposexual Behaviour

It was already demonstrated in early experiments in the 1940s that rats reared in isolation are either not capable to achieve ejaculation or remain sexually inactive, after repeated exposure to a receptive female (Beach 1942). In contrast, rats that were reared in groups with either same-sex or hetero-sex cage mates did not show these clear deficits in copulatory behaviour. Importantly, in most but not all of the

isolation-reared males, sexual performance gradually improved with experience. These early findings suggest that experience and learning play an important role in rat copulatory performance, but apparently do not exclusively determine the ability to successfully copulate until ejaculation. In early studies focussing on rats displaying different levels of sexual performance, in our laboratory we have tried to create hyposexual behaviour in male rats by manipulating the level of sexual experience (Mos et al. 1990). To this end, we have studied the sexual behaviour of 278 sexually naïve male Wistar rats in 15-min tests with an oestrus female. From those 278 males, 23 showed no sexual activity at all, i.e. no intromissions and maximally one mount was scored during the test. From the remaining 255 rats, 211 displayed sexual activity, but failed to ejaculate during the test. The average ejaculation latency of the 44 ejaculating males was 620 ± 28 s. If sexually naïve male rats were treated with 5-HT_{1A} receptor agonists, these males performed quite well (Table 1). In particular, the two full 5-HT_{1A} receptor agonists (±)-8-OH-DPAT and flesinoxan enhanced sexual behaviour to the level of sexually experienced male rats. The partial 5-HT_{1A} receptor agonists buspirone and ipsapirone also facilitated sexual activity. These findings indicate that naïve male rats are able to perform sexual activities reminiscent of sexually "experienced" rats in a very short time interval. Apparently, sexually naïve rats may be influenced by certain factors that can be overcome by treatment with psychoactive drugs, at least 5-HT_{1A} receptor agonists and (not shown here) α_2-adrenoceptor antagonists like yohimbine and idazoxan (Mos et al. 1990, 1991).

These pharmacological studies strongly suggest that neurobiological mechanisms underlie the differences observed in basal sexual behaviour.

3.8 Studies with Rats Displaying Hypersexual Behaviour

In contrast to studies focussing on rats that are hyposexual by nature, reports of rats that are hypersexual by nature are scarce. Nevertheless, numerous studies have indicated that a variety of selective pharmacological compounds, neurotransmitters and neuropeptides may facilitate sexual behaviour (Bitran and Hull 1987; Argiolas 1999). Most interesting are those studies in which male rat sexual behaviour is potently facilitated and in which the behaviour shares some of the characteristics of human premature ejaculation. Indeed, some of the clinical symptoms of premature ejaculation can be evoked pharmacologically in male rats. For instance, various selective 5-HT_{1A} receptor agonists have been shown to potently decrease ejaculation latencies and intromission and mount frequencies. Apart from selective 5-HT_{1A} receptor agonists, a selective dopamine D_2 receptor agonist SND-919 (Ferrari and Giuliani 1994) has also been shown to decrease ejaculation latencies in rats, although its effects were much less pronounced compared to the effects of 5-HT_{1A} receptor agonists.

Not only can pharmacological manipulations facilitate ejaculatory behaviour, but "tactile" stimulation, such as shock and tail-pinching (Barfield and Sachs 1968; Wang and Hull 1980), also facilitate ejaculatory behaviour. Presumably these

facilitatory effects are mediated by activation of the brain dopaminergic system (Leyton and Stewart 1996).

3.9 Conclusion: Serotonin and Male Sexual Behaviour

Research in humans and rats has indicated that modulating 5-HT levels in the CNS changes ejaculatory thresholds and associated sexual behaviour. Activation of 5-HT_{1A} receptors and blockade of 5-HT_{2C} receptors facilitates sexual behaviour, whereas activation of 5-HT_{1B} and 5-HT_{2A} receptors inhibits it. SSRIs, which facilitate serotonin neurotransmission, inhibit sexual behaviour but only after chronic administration or genetic inactivation of the SERT gene. There is a paucity of data on the putative role of other 5-HT receptors in the modulation of male sexual behaviour.

4 Serotonin, Serotonergic Receptors and Female Sexual Behaviour

4.1 Introduction

The pharmacology of sexual behaviour in females is rather restricted compared to males. The majority of work has focused on one aspect of it: the lordosis reflex. Female sexual behaviour consists of attractivity, proceptivity and receptivity. Attractivity reflects behaviour, smell and sounds by the female that attract the male and most often leads to proceptive behaviour of the female, including solicitation, hopping and darting. Receptivity is reflected in the lordosis reflex required for successful copulation. Beach (1948) introduced the lordosis quotient (LQ = lordosis to mount ratio X 100) reflecting the lordotic response of the female to a mounting male. The LQ is the most frequently used parameter when studying effects of hormones and drugs on female sexual behaviour (cf. Uphouse 2000; Uphouse and Guptarak 2010). The lordosis reflex (arching of the back, elevation of the rump, dorsoflexion of the tail and extension of the neck) is a very stereotyped posture in response to a mounting male (Pfaff 1999). The tactile stimulation stimulates cutaneous receptors in the flank, rump, tail base and perineum, which feed their information to the brain where primarily areas in the hypothalamus (notably the VMH) are crucial in the control of lordosis. Oestrogen (Er_{α}) receptor activation is required to induce the lordosis reflex, and there is a minimum amount of circulating oestrogen needed to reach a certain lordosis threshold. Moreover, a latent period (minimally 16 h) is needed for receptivity development. Normally, both oestrogen and progesterone are used to optimally organize the libido reflex, but progesterone is not needed if the oestrogen dose is extra high. Adding progesterone reduces the amount of oestrogen needed to induce lordosis behaviour.

Pharmacological studies often use submaximal oestrogen (or progesterone) doses in ovariectomized females which produce submaximal lordosis quotients and generate a model that can be pharmacologically manipulated. Early studies showed that reduction of monoamine levels in the brain (e.g. by pCPA or reserpine) activated lordosis in suboptimally oestrogen-primed ovariectomized rats, while activation of 5-HT function inhibits it (for review, see Uphouse 2000; Uphouse and Guptarak 2010). With the emerging availability of selective 5-HT receptor ligands more specific studies could be performed, but still serotonergic psychopharmacology has been mainly restricted to 5-HT_{1A} and 5-HT_2 receptors.

Activation of 5-HT_{1A} receptors leads to inhibition of the lordosis reflex in hormonally suboptimally and optimally primed female rats (Ahlenius et al. 1986; Mendelson and Gorzalka 1986). Work from Uphouse's group (Uphouse 2000) has found that the underlying mechanism of this inhibition is mediated via postsynaptic 5-HT_{1A} receptors in the hypothalamus, specifically, although not exclusively, in the VMH. Blocking of these 5-HT_{1A} receptors, however, did not lead to facilitation of the lordosis reflex which also does not happen after systemic administration of 5-HT_{1A} receptor antagonists (Uphouse 2000), a finding we confirmed in our laboratory (see SERT-KO data later).

The role of 5-HT_{1B} receptors in lordosis is somewhat disputed (Uphouse and Guptarak 2010). Notwithstanding the limited evidence and lack of selective agonists, data suggest that activation of presynaptic 5-HT_{1B} receptors facilitates lordosis (Mendelson 1992), whereas blockade of 5-HT_{1B} receptors inhibits it (Uphouse et al. 2009).

Activation of 5-$HT_{2A/2C}$ receptors (e.g. by DOI) facilitates lordosis in suboptimally primed rats (Mendelson and Gorzalka 1990), whereas 5-$HT_{2A/2C}$ receptor antagonists inhibit it (Hunter et al. 1985; Mendelson and Gorzalka 1985). These effects seem also to be mediated in the hypothalamus probably in close interaction with those mediated by 5-HT_{1A} receptors (Uphouse 2000; Uphouse and Guptarak 2010).

5-HT_3 receptors do not play an important role in female sexual behaviour; the few studies reported (for overview, see Uphouse and Guptarak 2010) do not point to central 5-HT_3 receptors as a primary target. Similarly, an inhibitory role in lordosis of 5-HT_7 receptors has been suggested (Siddiqui et al. 2007), but these data are much linked to 5-HT_{1A} receptor modulation and research involving selective 5-HT_7 receptor agonists is required.

As SSRIs are reported to induce a high incidence of sexual disturbance in human females (Balon 2006; Montgomery et al. 2002), it is relatively surprising that only a few studies have been performed in rats. Acute treatment with SSRIs reduces lordosis in hormonally primed ovariectomized rats (Frye et al. 2003; Sarkar et al. 2008). Because sexual side effects of SSRIs in humans are particularly disturbing after chronic administration, animal studies using chronic SSRIs are particularly relevant. Matuszcyk et al. (1998) found that chronic fluoxetine reduced sexual behaviour in female rats. This and other studies (Maswood et al. 2008; Uphouse and Guptarak 2010) are complicated by the fact that natural cycling females were used and fluoxetine affected the cycle, at least in a large number of the animals. A better strategy would be to chronically treat

ovariectomized female rats with an SSRI, prime them with a dose of oestrogen and progesterone to induce lordosis and to test the effects of the SSRI in this model. Sarkar et al. (2008) found, using this paradigm, that fluoxetine acutely reduced lordosis but this effect was attenuated after sub-chronic fluoxetine administration, suggesting that some tolerance for the sexual inhibitory effect of the SSRI had occurred.

4.2 SERT-KO Rats and Female Sexual Behaviour

An alternative way to study the role of the SERT in female sexual behaviour is using genetically modified animals, in this case the SERT-KO rat made by ENU mutagenesis (Smits et al. 2006). Female Wistar intact rats were tested in a paced mating design where sexually experienced males were restricted to one side of a cage, whereas the female (brought into behavioural oestrus by a high dose of oestradiol) could spend time on both sides of a divider which allowed passage of the female (but not the male) through a couple of openings in the divider. Figure 7 shows that mutant SERT genotypes ($SERT^{+/-}$ and $SERT^{-/-}$) were not different from wild types ($SERT^{+/+}$) in any aspect of proceptive or receptive behaviour over three consecutive tests of 30 min. This indicates that permanent absence of the serotonin transporter has no influence on female sexual behaviour under normal conditions. Treatment with a 5-HT_{1A} receptor agonist (+/−8-OH-DPAT) dose-dependently reduced proceptive behaviours (b) in all three genotypes, but in the

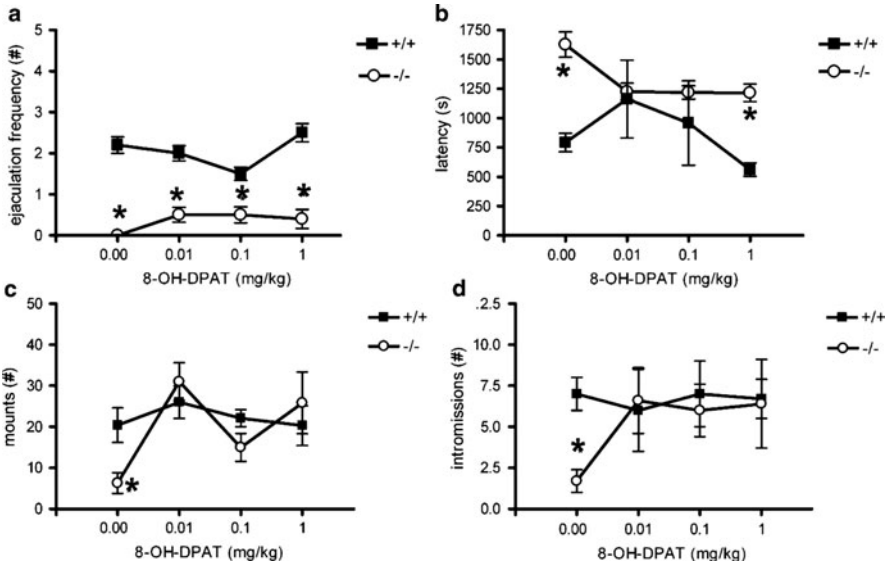

Fig. 7 Effects of three doses of 8-OH-DPAT (0.01, 0.1 and 1 mg/kg, SC) and one dose of WAY100635 (0.1 mg/kg, IP) on ejaculation frequency over 30-min test (**a**), latency to first ejaculation (**b**), first ejaculatory series mounts (**c**) and first ejaculatory series intromissions (**d**) of $SERT^{+/+}$ (+/+) and $SERT^{-/-}$ (−/−) animals. *$p < 0.05$ compared to wild type (+/+)

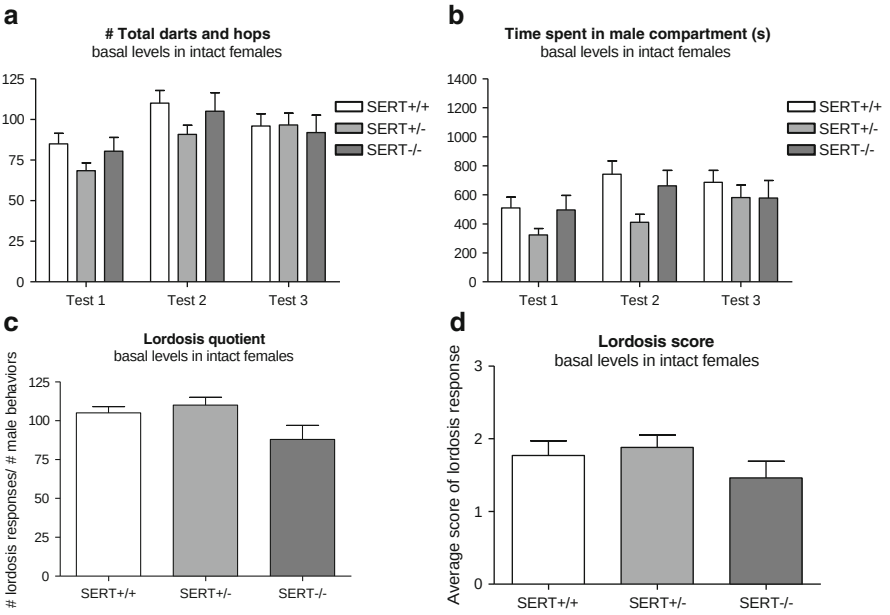

Fig. 8 In a paced mating situation (Snoeren et al. 2010) female wild-type ($SERT^{+/+}$), heterozygous ($SERT^{+/-}$) and homozygous ($SERT^{-/-}$) rats were brought into behavioural oestrus by hormonal priming and tested against a sexually experienced male rat. Females could pace the behaviour and stay in- or outside the male compartment. The number of proceptive [hopping and darting (**a**)] and receptive behaviours [Lordosis quotient and Lordosis score (**c** and **d**)] and the time spent in the male compartment (**b**) were measured

SERT-KO the dose–response curve clearly shifted to the right, indicative of a desensitized 5-HT_{1A} receptor (Fig. 8). However, time spent with the male was not affected (a), showing that the decreased proceptive behaviour was not caused by a diminished interaction with the male. Treatment with a 5-HT_{1A} receptor antagonist (WAY100635) did not affect any behaviour alone [(c) and (d)], whereas a selected dose of WAY100635 (0.1 mg/kg IP) was able to antagonize the 8-OH-DPAT-induced reduction in proceptive behaviour (f). Apparently, normal female sexual behaviour is not dependent on the functional status of 5-HT_{1A} receptors, but when challenged 5-HT_{1A} receptors appear desensitized in homozygous, but not heterozygous SERT-KO rats (Fig. 9).

5 Conclusions

The neurotransmitter serotonin clearly plays a role in male and female sexual behaviour (Table 2). Lowering serotonergic function seems to facilitate and enhancing it to inhibit sexual behaviour. The availability of blockers of the

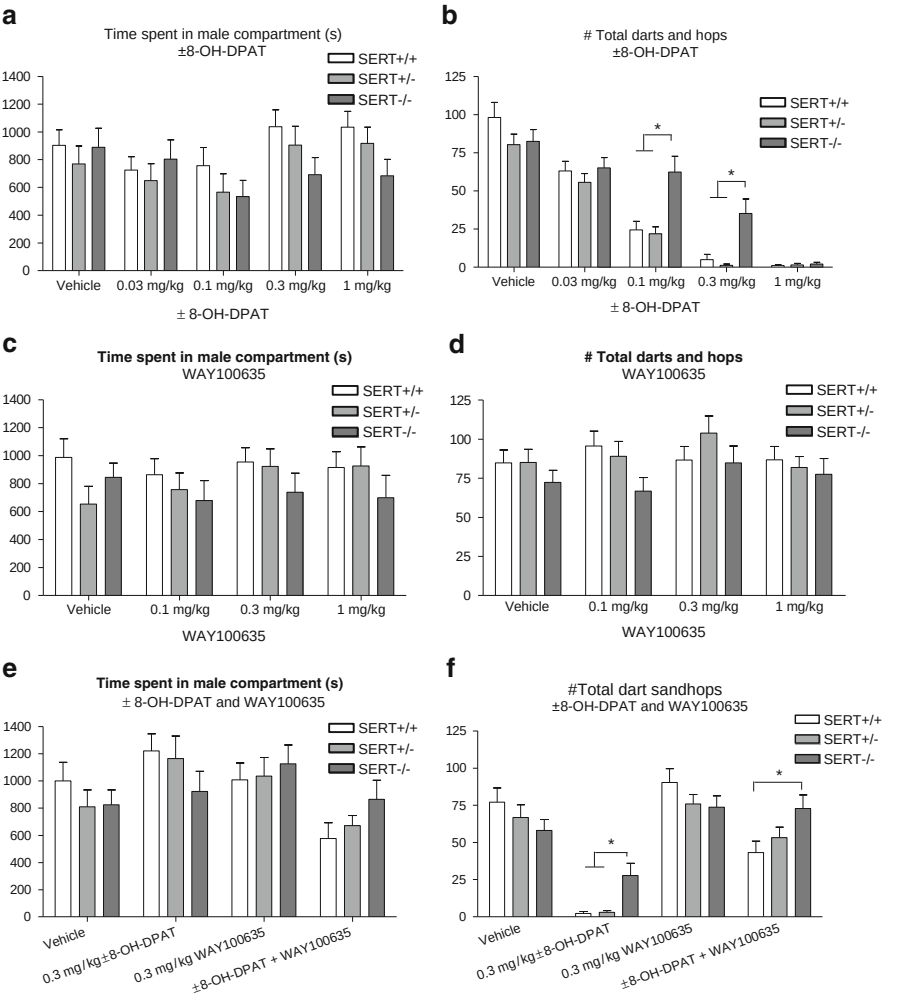

Fig. 9 Female wild-type ($SERT^{+/+}$), heterozygous serotonin transporter knockout ($SERT^{+/-}$) and homozygous serotonin transporter knockout ($SERT^{-/-}$) rats brought into behavioural oestrus were treated with the 5-HT_{1A} receptor agonist +/−8-OH-DPAT (**a**); the 5-HT_{1A} receptor antagonist WAY100639 (**b**) or a combination of selected doses of 8-OH-DPAT (0.3 mg/kg) and WAY100639 (0.3 mg/kg) (**c**). The *left part* of each figure shows the time spent by the female in the male compartment; the *right part* the number of proceptive behaviours (hopping and darting) performed by the female during the test. The test was performed using a paced mating design in which the male and female were separated by a perforated wall that could be crossed by the female but not by the male. The female decides whether she wants to spend time with the male and receive mounts, intromissions and ejaculations. $^{*}p < 0.05$ compared to wild type

Table 2 Summary of the effects of various serotonergic ligands on male and female sexual behaviour in rats after acute or chronic treatment

Target/ligand	Treatment	Male sexual behaviour	Female sexual behaviour
SERT/SSRI	Acute	=	=
SERT/SSRI	Chronic	↓	nd
5-HT_{1A} R agonist	Acute	↑	↓
5-HT_{1A} R agonist	Chronic	↑	nd
5-HT_{1A} R antagonist	Acute	=	=
5-HT_{1A} R antagonist	Chronic	nd	nd
5-HT_{1B} R agonist	Acute	↓	↑
5-HT_{1B} R agonist	Chronic	nd	nd
5-HT_{1B} R antagonist	Acute	=	↓
5-HT_{1B} R antagonist	Chronic	nd	nd
5-$HT_{2A/C}$ R agonist	Acute	↓	↑
5-$HT_{2A/C}$ R agonist	Chronic	nd	nd
5-$HT_{2A/C}$ R antagonist	Acute	↓	↓
5-$HT_{2A/C}$ R antagonist	Chronic	nd	nd
5-HT_7 R agonist	Acute	nd	↓
5-HT_7 R agonist	Chronic	=	nd
5-HT_7 R antagonist	Acute	=	=
5-HT_7 R antagonist	Chronic	=	nd

= not affected, *nd* not determined, ↑ enhanced, ↓ lowered, *R* receptor, *SSRI* selective serotonin reuptake inhibitor, *SERT* serotonin transporter

serotonin transporter and ligands for various serotonergic receptors has led to studies on male and female rat sexual behaviour that shed light on the contributions of individual receptors/transporter in male and female sexual function. SSRIs, blocking the SERT, generally lead to inhibition (after chronic treatment) of male and female sexual behaviour in agreement with the theory that enhancement of serotonergic function inhibits sexual behaviour. 5-HT_{1A} receptor activation facilitates male ejaculatory behaviour but inhibits female lordosis behaviour, suggesting an opposing role for this receptor in males and females. Clear-cut roles for other serotonergic receptors are less developed and need considerable research efforts.

Genetic manipulation of the SERT in rats indicated a differential influence of the absence of the SERT in male and female sexual behaviour; KO males, but not females, had lower baseline sexual activities. 5-HT_{1A} receptors were not desensitized in male KO, but were desensitized in females, indicating a differential role of various 5-HT_{1A} receptor pools in male and female sexual behaviour.

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Female Rats Are Smarter than Males: Influence of Test, Oestrogen Receptor Subtypes and Glutamate

Jane Suzanne Sutcliffe

Contents

Abstract Interest in the influence of sex hormones within the central nervous system is a rapidly expanding area of research. A considerable amount of evidence has recently been obtained to support an important role of the gonadal steroids in cognitive processing. Not only are distinct and complementary behavioural phenotypes evident for each gender, in the case of the female but they are also reliant upon hormonal status. Gender influences and hormonal status are thus paramount and should encourage the development of more hypothesis-driven research strategies to understand gender differences in both normal behaviour and where this is altered in neuropsychiatric disorders.

Keywords Cognition · In vivo · Oestrogen · Rat · Sex differences

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1 Introduction

Men and women differ not only in their physical attributes and reproductive function but also in many other characteristics including cognitive abilities and intellectual problem solving skills. For the past few decades, it has been ideologically fashionable to insist that these behavioural differences are minimal; research into sex differences in cognition has often been neglected and for many years, the male animal has been the standard gender used for behavioural research. Historically, female subjects have not been studied due to perceived problems inherent with oestrous cycle monitoring and also since it is assumed that their behaviour will be similar to those of a male. Strangely enough, evidence from human studies illustrates that men are actually more variable on tests of cognitive score and intellectual performance (Sahay and Hen 2007). Sex differences cannot be considered irrelevant as there are indisputable, and well-established gender differences in metabolism, neuroanatomy, endocrinology, biochemistry and behaviour (Cahill 2006). Moreover, sex differences in many behavioural paradigms have been reported since the early days of the last century (Baker 1987; Corey 1930). One of the most important discoveries regarding gender differences is concerned with the innate differences between the anatomy and neurophysiology of the male and female brain. Differences begin during early development due to a combination of genetic and hormonal events and persist throughout the lifespan of an individual [for a comprehensive review on the origins of gender differences, see (Wilson and Davies 2007)]. The imprinting of the female brain is not dependent upon androgen-oestrogen influence but for male differentiation, androgens must be present (Pilgrim and Reisert 1992).

Research during the past decade confirms that gonadal steroids, such as oestrogen, progesterone and testosterone, have the ability to influence the structural properties of the brain regions that sub-serve learning and memory. One of the most comprehensively characterised regions of the brain is the hippocampus, which has been shown to display different structural changes in response to different gonadal hormones. Evaluation of brain structure, function and chemistry over the course of the menstrual cycle as well as across the life span in women is critical to understanding sex differences in both normal and aberrant behaviour. This chapter provides an overview of common cognitive paradigms with respect to inherent sex-specific abilities and the impact of cyclic or manipulated hormonal changes on learning and memory in male and female rats. Mechanisms by which these gender specific abilities are purported to arise are also discussed with particular relevance to oestrogen receptors (ERs) and glutamate.

2 In vivo Evidence for Gender Differences in Cognition

The relationship between gonadal hormones and cognition remains immensely complex and depends on many factors, which require unique consideration such as the target brain structures and the recruited memory systems for that particular

cognitive challenge. Effects of the gonadal hormones and inherent sex differences on cognitive behaviour are not immediately obvious since gender differences are investigated across multiple laboratories, under different environmental conditions, with different strains of animals and sex differences are rarely discussed. Throughout this section, gender differences in popular cognitive paradigms are discussed and concisely compiled in Table 1.

2.1 Object Recognition Tasks

During the past few years, the ethologically relevant novel object recognition (NOR) and object displacement (OD) paradigms have enjoyed much scientific interest. These paradigms depend entirely on the rat's natural preference for novelty (objects and location), there is no requirement for rule specific learning and thus, there is a logistical advantage whereby the inherent variability during rule acquisition and undue stress is avoided. There is also good evidence to suggest that the NOR task is more sensitive to recognition memory impairments than other tasks such as the Delayed Non-Matching to Sample (DNMS) task (Nemanic et al. 2004; Pascalis et al. 2004). The NOR consists of a familiarisation phase, where two identical objects are presented to the subject, an inter-trial delay (which can be manipulated) and a test phase where one familiar and one novel object are presented to the subject (Ennaceur and Delacour 1988). Similar to the NOR task, the OD task consists of a familiarisation phase with presentation of two identical objects, which progresses, after an inter-trial delay, onto the test phase where one of these objects now occupies a new location with respect to the previous trial. Compared to incremental learning tasks using multiple learning trials, the NOR and OD tasks allow the investigation of drug effects on different stages of memory formation and recollection. To assess the influence of a compound on object encoding, it may be administered prior to the sample trial. The compound in question can also be administered intermediately (during the inter-trial interval) to assess the influence of drug effects on consolidation of object information. Thus, object recognition tasks are fast becoming a powerful ethologically relevant, scientific tool.

Recent research using object recognition tasks has provided clear evidence of gender-specific abilities (Sutcliffe et al. 2007). Both genders show a progressive decline when longer inter-trial intervals are experienced for both the NOR and the OD tasks; however, gender-specific cognitive abilities are evident. Female hooded-Lister rats have been shown to display a sustained object recognition memory when compared to male rats during this task when the length of memory retention is challenged. Recent studies (Sutcliffe et al. 2007) have demonstrated a clear female advantage during the NOR paradigm, where female hooded-Lister rats exhibit memory retention at inter-trial intervals of up to 3 h compared with 30 min in their male counterparts. The converse is true for the OD task, where male hooded-Lister rats exhibit a preference for the displaced object at an inter-trial interval of 3 h compared to only 30 min when compared to female hooded-Lister rats

Table 1 Summary of gender differences in common behavioural paradigms

Cognitive paradigm	Gender bias summary	Oestrous cycle influence	References
NOR	Females → Males Inconsistencies on actual length of memory retention across research groups	Cyclic performance, improved performance with high oestrogen and detrimental influence of ovariectomy which is reversed by hormone replacement	Sutcliffe et al. (2007), King et al. (2004), Ghi et al. (1999), Frye et al. (2007), Walf et al. (2006), Inagaki et al. (2010) and Aubele et al. (2008)
OD	Females ← Males	Cyclic performance, improved performance with high oestrogen	Sutcliffe et al. (2007) and Frye et al. (2007)
RAM/MWM	Small male advantage but inconsistent findings, often no sex differences observed	Negative effect of rising oestrogen across the oestrous cycle	Jonasson (2005), Bucci et al. (1995) and Faraji et al. (2010)
ZT	Females ← Males	Not reported	Faraji et al. (2010)
Social recognition	Young; Females → Males Aged; Females = Males	High oestrogen at pro-oestrous results in extended memory retention. Detrimental influence of ovariectomy, which is reversed by hormone replacement	Markham and Juraska (2007), Sánchez-Andrade and Kendrick (2011) and Hlinák (1993)
Eyeblink conditioning	Females → Males during acquisition only. Evidence suggests more females reach experimental criterion and retain the knowledge of the task longer than males	Detrimental influence of ovariectomy, which is reversed by supraphysiological levels of hormone replacement	Dalla et al. (2009), Wood and Shors (1998) and Leuner et al. (2004)
Fear conditioning	Females ← Males	Ovariectomy results in male-like performance ability	Maren et al. (1994), Pryce et al. (1999) and Gupta et al. (2001)
FR1	Females → Male during acquisition	No effect of ovariectomy	Dalla et al. (2008), Shors et al. (2007), Beatty and Beatty (1970) and Van Oyen et al. (1981)

(*continued*)

Table 1 (continued)

Cognitive paradigm	Gender bias summary	Oestrous cycle influence	References
FR2	Females → Male during acquisition	No effect of ovariectomy	Dalla et al. (2008), Shors et al. (2007), Beatty and Beatty (1970) and Van Oyen et al. (1981)
DRL/Lever pressing	Simple tasks Females ← Males ↑ Complexity Females → Male	Ovariectomy results in male-like performance ability	van Haaren et al. (1990), van Hest et al. (1987), Beatty (1973), Roth et al. (2004) and Lynch et al. (2002)
Self-administration	Faster acquisition in females	Ovariectomy results in male-like performance ability	Fattore et al. (2007, 2009), Roth et al. (2004) and Lynch et al. (2002)

(Fig. 1a–d) (Sutcliffe et al. 2007). Conversely, other research groups have demonstrated male hooded-Lister rats retain the preference for the novel object up to a 2 h inter-trial interval (King et al. 2004). Gender differences within the NOR paradigm have also been confirmed in the Wistar rat, females again exhibit superior performance during longer inter-trial intervals (90 min) when compared to their male counterparts (60 min) (Ghi et al. 1999).

Cyclic variations in sex hormones also exert a significant impact on performance during object recognition tasks. Female rats demonstrated a distinct variability in cognitive performance during the OD task throughout the oestrous cycle with enhanced object recognition during pro-oestrous or oestrous when compared to di-oestrous when oestrogen levels are at their lowest (Sutcliffe et al. 2007; Frye et al. 2007). No cyclic alteration in performance was observed during the NOR paradigm at a 1 h inter-trial interval (Sutcliffe et al. 2007), but a significant influence of the oestrous cycle during the NOR has been reported in female Long-Evans rats when the inter-trial interval is increased to a 4 h inter-trial interval, which was concomitant with increases in serum estradiol, progesterone and 3 alpha-hydroxy-5 alpha-pregnan-20-one (Walf et al. 2006). Further supporting the hypothesis that the sex steroids play an important role in cognition, ovariectomy produces a robust deficit in the NOR and OD paradigms, which is reversed with ovarian steroid replacement regimens (Inagaki et al. 2010; Frye et al. 2007. The cognitive deficit due to ovariectomy is a robust finding across research groups, in our laboratory sexually mature, ovariectomised animals were shown to significantly identify the novel from familiar object after an inter-trial interval of 1 h on weeks 1 and 2 but not weeks 3–6 and week 40 following surgery in comparison with sham-operated animals who retained recognition ability. Furthermore, ovariectomised animals receiving sub-cutaneous hormone replacement consisting of oestrogen alone or oestrogen in combination with a progestin (450 μg/kg 17-β-estradiol propionate once per week plus medroxyprogesterone 17-acetate or levonorgestrel at 15 mg/kg

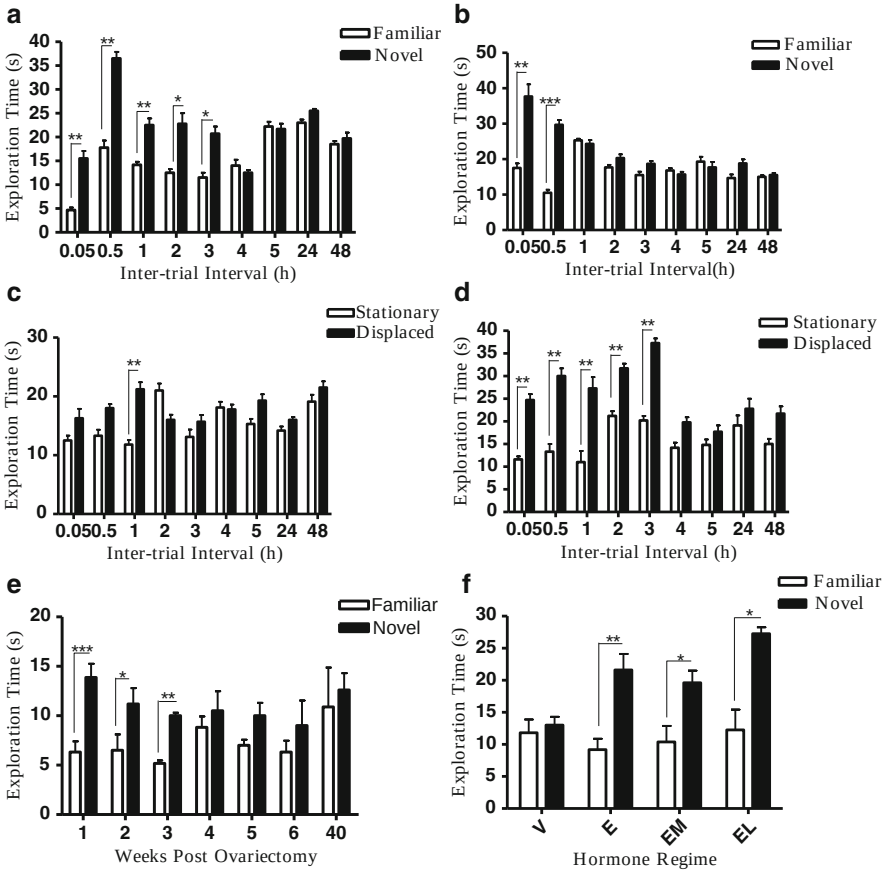

Fig. 1 Exploration times of gonadally intact mature female (**a**, **c**) and male (**b**, **d**) hooded-Lister rats during the retention trial in the NOR task (where one familiar and one novel object are presented to the subjects, **a** and **b**) and during the OD task (where two familiar, identical objects are presented to the subject but one object is displaced compared to the acquisition trial, **c** and **d**). Figures 1e and f illustrate the robust impact of ovariectomy (**e**) and continuous 16 week hormone replacement (**f**) – V (vehicle 1 ml/kg s.c), E (450 μg/kg 17-β-estradiol propionate s.c once per week), EM (E plus 15 mg/kg medroxyprogesterone 17-acetate s.c once every second week) and EL (E plus 15 mg/kg levonorgestrel s.c once every second week) during the NOR task with a 1h inter-trial interval. $*p < 0.05$, $**p < 0.01$ represent significant differences between the time spent exploring the familiar compared to the novel object. $**p < 0.05$, $**p < 0.01$, $***p < 0.001$ represent significant differences between the time spent exploring the displaced object compared to the stationary object. Data are expressed as the mean ± S.E.M. and were analysed at each time point using paired sample's *t*-tests ($n = 6$)

once every second week) for 15 weeks immediately after ovariectomy sustained NOR but not OD performance at a 1 h inter-trial interval. Delayed (initiation of hormone replacement at 13 weeks post-ovariectomy) and intermittent (initiation of treatment on weeks 1–6 resuming on 13–18) hormone replacement regimes

highlighted the benefit of initiating hormone replacement immediately following ovariectomy using a 1 h inter-trial interval in the NOR task. However, when the inter-trial interval was increased to 3 h, only the oestrogen alone intermittent group was able to significantly differentiate between the novel and familiar object (Fig. 1e, f. Sutcliffe et al., unpublished findings). Interestingly, gonadectomised (GDX) mature male Sprague-Dawley rats explore a novel and familiar object equally during the NOR task, and these adverse effects of gonadectomy are attenuated by supplementing GDX animals with testosterone-propionate but not 17-β-estradiol (Aubele et al. 2008). Endogenous or exogenous oestrogen replacement is consistently reported to be beneficial during the NOR task for females, a finding which is not reported in males, suggesting innate activational differences by which oestrogen acts within the brain.

2.2 Spatial Maze Paradigms

The existence of gender differences in rodent models of spatial learning and memory is a prominent yet controversial and often contested topic in the literature. A variety of studies have revealed a male superiority during performance on spatial tasks such as the Radial Arm Maze (RAM) developed by Olton and Samuelson (Olton and Samuelson 1976) and the Morris Water Maze (MWM) developed by Roger Morris (Morris 1981). In the MWM task, subjects will swim in a pool of water to identify/locate a hidden platform employing the topographical relationships among the distal visual cues, pool wall and goal location. Similar to the MWM, subjects completing the RAM must find the locations of food rewards at the end of some of the maze arms and retrieve this information for successive trials. The RAM has primarily been adopted to assess spatial working memory but is also adaptable to recruit working and reference memory simultaneously. During both the standard 8-arm version and the 17-arm version of this task, gender differences have not always been reliably demonstrated with some inconsistent findings (Jonasson 2005).

A direct comparison between male and female Long Evans rats at 6 months of age in the MWM task has concluded that there are no sex differences in place learning ability. Furthermore, search accuracy on probe trials, when the platform was unavailable, was also equivalent for the male and female groups. A recent review illustrates that, while there is a distinct male advantage on spatial tasks there is a lack of reliable replication across different laboratories which may be due to the strain of animal used, variations in stage of the oestrous cycle and stress levels whilst under test (Jonasson 2005). Fisher, Long Evans and Sprague-Dawley rats have been shown to yield the largest and most robust male advantage during spatial cognition tasks, while the same tasks employed in Wistar rats denote only a small male advantage when compared to female performance (Bucci et al. 1995). Interestingly, the promising development of a new paradigm – the dry-land ziggurat task (ZT) may provide the research community with more robust and consistent

gender-related results. The ZT consists of an open field containing 16 identical ziggurats (pyramid-shaped towers) positioned equal distances apart. One ziggurat is baited with a food reward and the rat must navigate through the open field to retrieve the food reward using a combination of distal and/or proximal cues. The ZT relies on the ability of the test subject to acquire and recall the location of the baited ziggurat and this is tested in consecutive training sessions of eight trials per day for 10 days. The location of the baited ziggurat is changed every second day, requiring the rats to learn a total of five different locations. Indices of learning and memory are based on several parameters, including latency to find the target, distance travelled, the number of visits to non-baited ziggurats (errors) and the number of returns. A recent study directly compared the performance of male and female Long-Evans rats in the wet-land MWT with the dry-land ZT. While males and females did not display significant differences in the traditional measures of spatial navigation within the MWT, they displayed a robust, male biased, sex difference in all measures of the ZT indicating task-specific gender differences in spatial performance. Taken together these findings suggest that males and females may employ different learning strategies in the MWT and ZT and that the latter task provides a more favourable task for assessing gender differences in spatial memory in rats (Faraji et al. 2010).

Fluctuations in sex hormones (e.g., oestrogen, progesterone and testosterone) will undoubtedly cause a shift in male and female performance. Varying levels of oestrogen results in variations in spatial learning and memory so that, when tested across the oestrous cycle females perform as well as males on days of low oestrogen but poorly when levels rise (Frye 1995; Warren and Juraska 1997). When female Long Evans rats are tested at a single point during the oestrous cycle, females in oestrous outperform those during the pro-oestrous phase (Warren and Juraska 1997). Conversely, other studies have found limited or no cyclical variations in performance (Healy et al. 1999; Bucci et al. 1995; Berry et al. 1997), indicating that retention for spatial information may be preserved despite morphological alterations in hippocampal dendritic spine density in the normally cycling female rat. Interestingly, administration of chronic oestrogen to ovariectomised rats enhanced spatial memory during the RAM task (Luine et al. 1998) and age-associated decline in sex steroid levels have been found to more profoundly impair spatial working memory in female rats in comparison with their male counterparts (Markowska 1999). Taken together, this evidence suggests a pivotal role for oestrogen for mnemonic abilities.

Variations in stress levels of the animals whilst under testing conditions may also play a critical role in gender-specific abilities during spatial paradigms. A growing body of literature demonstrates that certain aspects of spatial mnemonic function are dependent upon a stress response pattern, which is shown to be sexually dimorphic. The deleterious influence of chronic stress evident in male subjects in numerous spatial tasks, displayed as an impaired performance is not evident in female rats and this effect is thought to be mediated by oestrogen (to be discussed in more detail later in this chapter, Sect. 3).

2.3 Social Recognition

Social recognition memory, vitally important for social interaction and the establishment of dominance hierarchies, is the ability to discriminate between unfamiliar and familiar conspecifics. In rodents, the task utilises chemosensory cues present in the anogenital region, which composes an "olfactory signature". Similar to the NOR task, the amount of time one individual spends investigating another may be evaluated in the laboratory. Young (3–5 months) adult female rats have been shown to discriminate between novel and familiar juveniles for longer intervals than males (a 120 min compared to a 90 min interval), although aged male and female rats (16.5–19.5 months) have been shown to display the same social discrimination memory abilities (Markham and Juraska 2007). The superior memory ability of females was not found to be preserved during ageing, discrimination between a novel and familiar juvenile was abolished after a 120 min interval.

More recently, the influence of gender and the oestrous cycle has been evaluated on the formation and long-term (24 h) maintenance of social recognition memory in mice with a focus on the respective involvement of α- and β-oestrogen receptors. Female wild-type animals were able to successfully form memories during all stages of their oestrous cycle however, only when learning occurred during proestrus (when oestrogen levels are highest) was the memory retained for a period of 24 h. The acyclic, α-receptor knockout female mice demonstrated impairments in both the formation and the maintenance of social recognition memory, whereas β-receptor female knockouts showed no significant deficits and exhibited the same proestrus-dependent retention of memory at 24 h akin to the wild-type mice. To investigate gender differences, male α- and β-oestrogen receptor knockout mice were also evaluated in the same paradigm demonstrating similar results to the females. The male α-receptor knockouts had normal memory formation and only exhibited the 24 h memory retention deficit, indicating a greater female dependence on α-receptor expression for memory formation during this specific task (Sánchez-Andrade and Kendrick 2011).

Historically, the behavioural phenomenon of social recognition has been extensively studied in males. Insights into the impact of hormonal states on recognition memory (towards a juvenile male) in females have demonstrated that in young adult female rats social recognition memory is negatively affected 3 weeks after ovariectomy and restored with oestrogen replacement, co-incidentally, similar those deficits observed during the NOR task (Fig. 1e, f. Sutcliffe et al., unpublished findings. Complementing these findings it was further observed that 6 weeks after the termination of oestrogen replacement recognition memory was once again impaired (Hlinák 1993).

2.4 Classical and Operant Conditioning

The classical, hippocampal-dependent, trace eyeblink conditioning paradigm is viewed as an associative task in which the animal is presented with a conditioned

stimulus (normally white noise) followed closely by an aversive eyelid stimulation, the result of which causes the animal to blink (an unconditioned response). As the animal learns, the unconditioned response becomes a conditioned response (i.e., the animal learns to predict the eyelid stimulation in advance). Prior to training, age-matched male and female Sprague-Dawley rats express similar levels of spontaneous blinking; however, females learn to anticipate the onset of the unconditioned stimulus and thus learn to time the conditioned response (i.e. eyeblink) sooner than males, this response being most evident on the first day of training. At the end of training, there are no gender differences but interestingly, more females than males reach a criterion of 60% conditioned responses (7 out of 8 females compared to 7 out of 10 males reaching the same criterion) (Dalla et al. 2009). Perhaps not surprisingly, re-exposure of trained animals to the conditioned stimulus some weeks later elicits a higher percentage conditioned response in females compared to the males suggesting that the female rats have learnt and retained the rules of this task better than the males (Dalla et al. 2009). Further demonstrating oestrogen sensitivity in this task, removal of the ovaries prevents the sex differences in performance (Wood and Shors 1998) but enhanced conditioned responding is displayed in ovariectomised female rats following two injections of 40 μg estradiol 24 h apart, albeit at supraphysiological doses which produced plasma estradiol levels of greater than 250 pg/mL (Leuner et al. 2004).

Sex differences have also been reported in another type of classical conditioning referred to as fear conditioning. Typically, the animal is trained to associate a cue or a context (e.g., a tone) with an aversive stimulus (i.e. a footshock). Re-exposure to the same cue or context results in the animal "freezing" or to express an enhanced startle reflex in anticipation of the aversive stimulus. A male bias is typically observed during cue fear conditioning since male rats acquire the association between the cue and foot shock quicker than females (Maren et al. 1994). The ability of males to outperform females within this paradigm is also a consistent finding in three strains of laboratory rat, the Wistar, Fischer and Lewis (Pryce et al. 1999). Castration elicits no effect on the conditioned response (Anagnostaras et al. 1998); in contrast, ovariectomy results in female rats displaying comparable levels of fear to males (Gupta et al. 2001).

Although in classical fear conditioning studies males have outperformed the females, during more complex avoidance operant tasks the opposite is true. During an operant task, the animal must make an overt response in order to learn, which is often to escape an aversive stimulus – normally a mild foot shock. During the one-way avoidance task (FR1), the animal must learn to pass through the door way of a shuttle box once to avoid a mild footshock (FR1). This task is often learnt within a day and it has been observed that female rats will learn this task sooner than the males (Dalla et al. 2008; Shors et al. 2007). When the task difficulty is increased to the two-way avoidance task (FR2) where the animals must learn to pass through the doorway twice to terminate the footshock, more striking gender differences have been observed (Dalla et al. 2008; Shors et al. 2007). Females acquire the FR2 task within the first few trials but males require more trials and in some cases never learn the rule (Dalla et al. 2008). It would appear that female rats respond actively

to aversive stimuli, whereas males exhibit behavioural inhibition with passive reactions and freezing. Ovary removal does not prevent the gender difference in operant conditioning (Beatty and Beatty 1970; Van Oyen et al. 1981); however, cyclic oestrogen and progesterone influence conditioned avoidance behaviour and escape latencies as evidenced by enhanced performance during pro-oestrous when oestrogen levels are at their highest (Sfikakis et al. 1978).

Another form of operant conditioning which has shown sexual dimorphism is differential-reinforcement-of-low-rate of responding (DRL), a task in which the animal must learn to press a lever for a reward (often food). Irrespective of reward receipt, male animals have shown better performance when compared to females during acquisition of instrumental responding, which is contributed to a higher level of interaction with the lever (van Haaren et al. 1990). On the contrary, when the task difficulty is more complex and the animals have to systematically increase the number of times they press the lever to receive the reward, females outperform the males (van Hest et al. 1987) – although incentive motivation for the reward could be a driving factor. Gender differences in differential reinforcement paradigms are also reliant upon the activational effects of the gonadal hormones, since they are not observed during puberty and are abolished with ovariectomy (Beatty 1973).

Finally, sex differences are also observed in stimulant self-administration paradigms. Female rats are consistently reported to be more sensitive to the reinforcing actions of stimulants and acquire stable, high levels of drug self-administration of low doses of cocaine, methamphetamine, opioids and nicotine at a faster rate than males [for comprehensive reviews see (Fattore et al. 2009; Roth et al. 2004; Lynch et al. 2002)]. Ovariectomy results in slower cannabinoid self-administration acquisition when compared to cycling Long-Evans and Lister-hooded rats. Intriguingly, in these rat strains ovariectomy decreased cannabinoid intake to the same level as those shown by males (Fattore et al. 2007, 2009), suggesting that the ovarian hormones play a crucial role in these responses to cannabinoids.

3 Female Advantage Under Stressful Conditions

The limbic region, critical for processing information, shows plasticity under chronic stress with important gender-related differences (Eichenbaum et al. 2007; Lipton and Eichenbaum 2008). Chronic stress has repeatedly been shown to impair spatial learning and memory in male subjects yet produces different outcomes in females, thus gender is gaining recognition as an important variable acting as a mitigating factor or fundamental aetiology influencing stress-related disorders. Male rats exposed to chronic stress (6 h daily restraint for 21 days) perform poorly on tasks where normally a common male advantage is observed such as the RAM (Luine et al. 1993, 1994; Bowman et al. 2003), Y-maze (Conrad et al. 1996; Wright and Conrad 2005) and the MWM (Markowska 1999). In an almost opposite outcome, stressed female rats perform and complete the maze task with fewer

errors and more correct choices (30% enhancement) when compared to unstressed females (Bowman et al. 2003). Not only does this female advantage influence classical tasks for spatial memory, stress has also been demonstrated to enhance female rat performance during the object placement task at an ITI of 2.5 h and 4 h (Beck and Luine 2002), where unstressed females are unable to perform this task at ITIs of greater than 1 h.

Dendritic retraction of the CA3 region of the hippocampus has been implicated in the observed sexual dimorphisms under stressful situations. Chronic stress administered to cycling female rats has been shown to result in mild (Galea et al. 1997) or no (McLaughlin et al. 2010) dendritic retraction in the CA3 region. In ovariectomised rats, chronic stress produces drastic CA3 dendritic retraction (McLaughlin et al. 2010); however, spatial learning and memory remains functional and is even facilitated in ovariectomised and cycling chronically stressed female rats. This disconnection between the CA3 dendritic retractions and spatial memory in females may be attributed to the ovarian hormones. Indeed, within the CA3 region, females express more ERβ immunoreactivity when compared to the CA3 region in male rats (Zhang et al. 2002), implying that oestrogens may be the key neuroprotective agent to prevent stress-induced CA3 dendritic attrition.

4 Mechanisms of Sex Hormone Action for Cognition? The Oestrogen Perspective

Much evidence now supports a role for the interplay of many neurotransmitter systems, neuroplasticity and oestrogen with respect to understanding cognitive abilities. Many of oestrogen's actions in the brain are attributed to the activation of the classical ERs α and β (ERα and ERβ, respectively) and their subsequent impact on synaptic density and morphology, which is concomitant with alterations in learning and memory.

4.1 Hippocampal Architecture

The medial temporal lobe system, including the hippocampal formation (entorhinal cortex, dentate gyrus, areas CA1-4 and subiculum), amygdale, and the parahippocampal cortices are considered to serve as a declarative memory system. The hippocampal formation, along with the frontal cortex, is an extensively explored area of the brain with regard to cognitive competence. This region has been investigated by means of excitotoxic, ablation and radiofrequency lesions, transient neuronal inactivation (lidocaine), lesions, or transections of its connections, by pharmacological N-methyl-D-Aspartate (NMDA) receptor blockade and even via genetic inactivation of the CA1-NMDA receptors (Dere et al. 2007) pre-clinically – evidence of which is supported by

human lesion studies (Zola-Morgan et al. 1986). Cytotoxic and radio-frequency lesions to the hippocampus have been shown to result in an object recognition impairment after inter-trial intervals of 5 and 10 min, 1, 4 and 24 h (Ainge et al. 2006; Clark et al. 2000; Mumby et al. 2002). However, the size of the lesion to the hippocampus (>75%) remains important for object recognition memory (Broadbent et al. 2004). Oestrogen-associated changes (direct or indirect) within the hippocampus impact upon neural pathways, acting to alter performance in specific tasks. In the hippocampus of the female rat, Woolley and colleagues (Woolley et al. 1990; Woolley and McEwen 1992, 1994; Woolley et al. 1997; Woolley 1998) discovered that CA1 spine density naturally fluctuates across the female rat oestrous cycle, peaking with an increase of 32% during pro-oestrus when oestrogen levels are at a maximum compared to the di-oestrus phase. Subsequent studies have shown that oestrogen replacement in the ovariectomised female rat increases CA1 spine density (Woolley and McEwen 1992, 1993; Woolley 1998; Birzniece et al. 2006) through NMDA receptor-mediated mechanisms (Woolley and McEwen 1994; Woolley et al. 1997) and ERβ activation (Liu et al. 2008). This research may provide important mechanistic evidence for the female advantage in object and social recognition tasks; furthermore, neuroprotection is observed when oestrogen is administered prior to the NMDA antagonist, PCP (Sutcliffe et al. 2008). This important relationship between glutamate, NMDA and oestrogen is discussed in more detail in Sects. 4.3 and 4.4.

4.2 Oestrogen Receptors

Sex differences exist in the majority of brain regions including many involved in cognitive function such as the hippocampus, amygdala and neocortex (Juraska 1991). In many cases, these gender differences are not evident in overt anatomical structure, but in functional dimensions (e.g., a brain region may differ between the sexes in aspects of neurotransmitter function). The majority of research has investigated the effect of the steroid oestrogen on cognitive function and ERs have been found in several areas of the brain including the amygdala, cerebral cortex, cerebellum and hippocampus (Shughrue and Merchenthaler 2000; Tsutsui et al. 2004). ERs are important not only for the sexual differentiation of the brain but also are known to exert receptor-mediated functions on a number of behavioural functions. While the existence of ERα has been long known (Jensen et al. 2010), the discovery of ERβ has been much more recent (Kuiper et al. 1996). Of particular interest to the present topic is the hippocampus which is known to play a significant role in working and spatial memory (Jarrard 1993) and which expresses both forms (α and β) of the ER (Cahill 2006; Frye 1995; Birzniece et al. 2006); however, their distribution does not completely overlap (Milner et al. 2001, 2005). ERβ is the predominant ER in the cerebral cortex and the hippocampus. Other areas within the brain which are confirmed as supporting mnemonic processes include the frontal cortex and the striatum, and it has become clear that there are strong interconnections (or loops) between the hippocampus

Table 2 Distribution and gender differences in ERβ immunoreactivity in the Wistar rat brain

Region of interest	Female	Male
CA1	+/−	+/−
CA2	+/−	+/−
CA3	++	+
CA4	++	+
Dentate gyrus	++	+
Endopiriform nucleus	+++	+++
Medial septal nucleus	+++	++
Purkinje cells	+++	+++
Lateral and medial amygdaloid	++	+

+++, high; ++, moderate, +, low and +/−, weak.
Source: Shughrue et al. (2000)

and frontal cortex (Vertes 2006). In the frontal cortex of male and female rats, each ER isoform has been shown to display its own unique, selective distribution (Zhang et al. 2002; Kritzer 2002). Expression of ERβ in the medial mammillary nucleus (a limbic area often associated with the hypothalamus) was only detected in the male rat brain. The female rat brain shows a higher predominance of ERβ immunoreactivity (Table 2) in the medial septal nucleus (an area which receives reciprocal connections from the olfactory bulb, hippocampus, amygdala and hypothalamus), pyramidal cells of the CA3 and CA4, the dentate gyrus and the lateral amygdaloid nucleus (Shughrue et al. 2000). Whether such gender differences in anatomical expression occur across species and in man has yet to be shown but the development of novel ER molecules as therapeutic agents remains an exciting prospect, especially considering the growing body of literature suggesting ERβ modulation regulates neuroplasticity and cognition (Liu et al. 2008; Choleris et al. 2008; Walf et al. 2008; Rhodes and Frye 2006).

4.3 Neurotransmission

The ability of oestradiol to induce and increase dendritic spine density is suggested to be due to the reduction of GABA inhibition by oestradiol in the hippocampal area (Murphy et al. 1998; Weaver et al. 1997). Furthermore, this oestrogen-induced increase in spine density is positively correlated with increases in NMDA receptor binding and sensitivity (Daniel and Dohanich 2001) providing one possible mechanism for the ability of oestrogen to improve cognitive function, especially when administered prior to an NMDA receptor antagonist such as PCP (Sutcliffe et al. 2008). Ovariectomy results in significant decreases in choline acetyltransferase (ChAT) activity and high affinity choline uptake (HACU) in the rat basal forebrain, hippocampal formation and cerebral cortex beyond normal ageing (Gibbs et al. 2002), which can be reversed by acute treatment with physiological levels of oestrogen [10 μg/kg oestradiol benzoate (Luine 1985; O'Malley et al. 1987) and

17-β-estradiol silastic capsules (Singh et al. 1994)]. Thus, oestrogen (exogenous or endogenous) may play an important role in the maintenance or regeneration of healthy cholinergic projections to the hippocampus and prefrontal cortex, which, in turn may be correlated with the enhanced cognitive performance observed during some cognitive paradigms when compared to males. One of the mechanisms by which oestrogen may enhance cognitive function is the modulation of the production and release of acetylcholine (ACh), which is shown to enhance the amplitude of synaptic potentials following long-term potentiation in regions such as the dentate gyrus, CA1, piriform cortex, and neocortex. This effect most likely occurs either through an indirect action on NMDA receptors, which are shown to be more abundant in the CA1 region of the female hippocampus compared to the male.

Work in understanding how oestrogen affects cognition has concentrated on the oestrogen-mediated changes in hippocampal spine density and synaptogenesis favouring an NMDA-dependent mechanism of GABAergic disinhibition of pyramidal neurons mediated through non-genomic actions. Recent reports indicate that septal cholinergic inputs are required for this oestrogen-mediated enhancement of working memory and NMDA receptor binding in the CA1 region of the hippocampus (Daniel and Dohanich 2001) and oestrogen-mediated changes in hippocampal spine density in rats (Lam and Leranth 2003), thereby linking these mechanisms.

4.4 Glutamate and Oestrogen

Glutamate is the predominant excitatory amino acid neurotransmitter in the cortical, hippocampal and hypothalamic areas of the brain (Brann 1995) and glutamate receptors are considered to be responsible for the glutamate-mediated post-synaptic excitation of neural cells – important for neural transmission, memory consolidation through synaptic plasticity and accordingly learning. Synergistic interactions between glutamate and the gonadal steroids may underlie multiple limbic roles. The two primary glutamate receptors are NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), named after their preferred agonists, and it is through the NMDA and AMPA receptors that glutamate may be neurotoxic. Glutamate toxicity may be predominantly mediated by the NMDA subclass of glutamate receptors (Choi et al. 1988). Administration of the non-competitive NMDA receptor antagonist, PCP, has reliably been shown to result in cognitive deficits in the rat (Neill et al. 2010) after both acute and sub-chronic administration, such deficits have been reversed with both the atypical antipsychotics (Grayson et al. 2007) and after pre-treatment with oestradiol-benzoate (Sutcliffe et al. 2008). Protective effects of oestrogens against glutamate toxicity have been described in cultured hippocampal neurons (Goodman et al. 1996). The toxicity experienced by each hippocampal culture was attenuated following a 2 h pre-treatment with 100 nM to 10 mM 17-β Estradiol, estriol or progesterone. This protective influence of oestrogen is further confirmed by the finding that a 24 h pre-treatment with 15–50 nM of 17-β Estradiol significantly decreased the lactate dehydrogenase efflux from primary cortical neurons exposed

to glutamate for 5 min (Singer et al. 1996, 1999). Moreover, it was demonstrated that the selective ER modulator, tamoxifen, blocked the protective effects of oestrogen, thus suggesting that classical ER activations are required for oestrogen neuroprotection against glutamate toxicity. Accumulating evidence suggests a sexually dimorphic vulnerability to neurological insults with the ER receptors conveying differential protective capacities (Bryant and Dorsa 2010). Accordingly, a therapeutic opportunity exists for oestrogen modulators, not only in the acute reversal of disease-induced cognitive deficits but also for long-term neuroprotection in neurodegenerative disease.

5 Summary and Conclusions

Gender differences in cognition are evident in both pre-clinical models and in the clinic. Gender differences in learning vary as a function of the demands of the task and this review has primarily focussed on those where a female bias is observed. However, there are paradigms in which males excel such as those which require spatial navigation. In recent years, research in the area surrounding hormonal function in the central nervous system has increased with new discoveries and technology aiding the advance of knowledge regarding the effects of hormones on neurotransmission and consequent interactions within the brain. Despite these advances, research is still only in the early stages and suggests the complex nature of these systems with more questions being raised than answered. Only by increasing our understanding of gender differences with respect to learning and memory we can improve the translational value of our pre-clinical models in order to prevent and more effectively treat cognitive alterations associated with the aetiology and symptomatology of psychiatric and neuropsychological disorders.

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Sex Differences in the Septo-Hippocampal Cholinergic System in Rats: Behavioral Consequences

Dai Mitsushima

Contents

Abstract The hippocampus is processing temporal and spatial information in particular contexts or episodes. Using freely moving rats, we monitored extracellular levels of acetylcholine (ACh), a critical neurotransmitter activating hippocampal circuits. We found that the ACh release in the dorsal hippocampus increases during the period of learning or exploration, exhibiting a sex-specific 24-h release profile. Moreover, neonatal increase in circulating androgen not only androgenizes behavioral and hormonal features, but also produces male-type ACh release profile after the development. The results suggest neonatal sexual differentiation of septo-hippocampal cholinergic system. Environmental conditions (such as stress, housing or food) of animals further affected the ACh release.

Although recent advances of neuroscience successfully revealed molecular/cellular mechanism of learning and memory, most research were performed using

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male animals at specific time period. Sex-specific or time-dependent hippocampal functions are still largely unknown.

Keywords Acetylcholine · Androgen · Diurnal rhythm · Estrogen · Learning and memory · Sex difference

1 General Introduction

The hippocampus is a part of the limbic system and is a brain structure critically involved in learning and memory. The hippocampus processes temporal and spatial information within specific episodes (Komorowski et al. 2009; Gelbard-Sagiv et al. 2008). In freely moving animals, acetylcholine (ACh) release in the hippocampus increases during learning or exploration, showing a temporal 24-h release profile. ACh release changes with spontaneous movement (Day et al. 1991; Mitsushima et al. 1996) that stimulates electrical activity of cholinergic neurons in the basal forebrain (Buzsáki et al. 1988). Moreover, voluntary running enhances learning in mice (van Praag et al. 1999), while a restriction of exploratory behavior impairs ACh release and learning (Mitsushima et al. 1998, 2001). In this review, we focused on in vivo ACh release in the hippocampus to improve our understanding of the role of sexual dimorphism and temporal effects on hippocampal function.

2 Physiological Role of ACh in the Hippocampus

A number of studies suggest that ACh plays an important role in orchestrating major hippocampal functions (Fig. 1). In behavioral studies, ACh release increases during learning (Ragozzino et al. 1996; Stancampiano et al. 1999; Hironaka et al. 2001) and is positively correlated with learning performance (Gold 2003; Parent and Baxter 2004). Bilateral injections of scopolamine into the dorsal hippocampus impair spatial learning ability (Herrera-Morales et al. 2007), suggesting that muscarinic ACh receptors mediate the formation of spatial memory. At the network level, ACh generates a theta rhythm (Lee et al. 1994) that modulates the induction of long-term potentiation (LTP) in hippocampal CA1 neurons (Hyman et al. 2003). Studies exploring a genetic deficiency of muscarinic ACh receptors (M_1 or M_2) further show the impairment of LTP in the CA1 region (Seeger et al. 2004; Shinoe et al. 2005). At the cellular level, both pyramidal and nonpyramidal neurons in the hippocampal CA1 area receive direct cholinergic afferents mediated by muscarinic receptors (Cole and Nicoll 1983; Markram and Segal 1990; Widmer et al. 2006). In vitro studies showed that bath application of carbachol, a cholinergic agonist, induces LTP in CA1 pyramidal neurons without electrical stimulus,

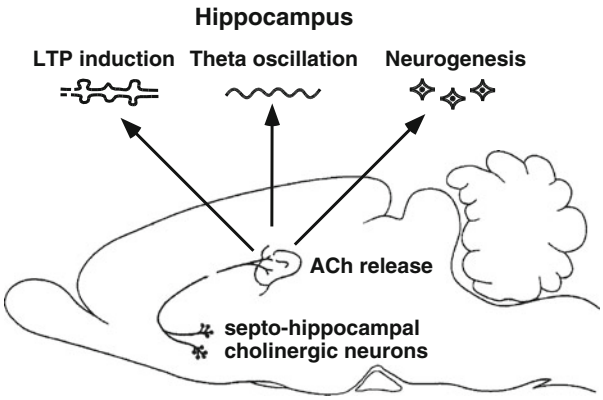

Fig. 1 Schematic illustration of septo-hippocampal cholinergic neurons. The released ACh activates major hippocampal functions. ACh, acetylcholine. LTP, long-term potentiation

suggesting that ACh in the hippocampus plays a principal role in the synaptic plasticity of the CA1 pyramidal neurons (Auerbach and Segal 1996). Furthermore, a recent study revealed an intracellular mechanism of ACh: focal activation of muscarinic ACh receptors in one CA1 pyramidal neuron induces Ca^{2+} release from inositol 1,4,5-trisphosphate-sensitive stores to induce LTP (Fernández de Sevilla et al. 2008). Furthermore, not only is ACh critically involved in synaptic plasticity, but ACh release in the hippocampus is also responsible for neurogenesis in the dentate gyrus. Thus, neurotoxic lesions of forebrain cholinergic neurons or long-term scopolamine treatment significantly decreases the number of newborn cells in the dentate gyrus, approximately 90% of those were also positive for the neuron-specific marker NeuN (Mohapel et al. 2005; Kotani et al. 2006).

3 Monitoring of In Vivo ACh Release

Cholinergic neurons within the basal forebrain provide the major projection to the neocortex and hippocampus (Mesulam et al. 1983). Cortical regions receive cholinergic inputs mainly from the nucleus basalis magnocellularis (NBM) or the diagonal band of Broca, whereas the hippocampus receives cholinergic inputs mostly from the medial septum and horizontal limb of the diagonal band of Broca (Mesulam et al. 1983). Because the cholinergic projections are necessary to maintain learning and memory (Perry et al. 1999, Sarter and Parikh 2005), we hypothesized that in vivo monitoring of ACh release in the hippocampus is necessary to elucidate learning function. To measure ACh release, we have performed in vivo microdialysis studies in freely moving male rats. Briefly, a microdialysis probe with a semipermeable membrane (1.0 mm in length) was inserted into a specific brain area via a surgically pre-implanted guide cannula. We perfused the

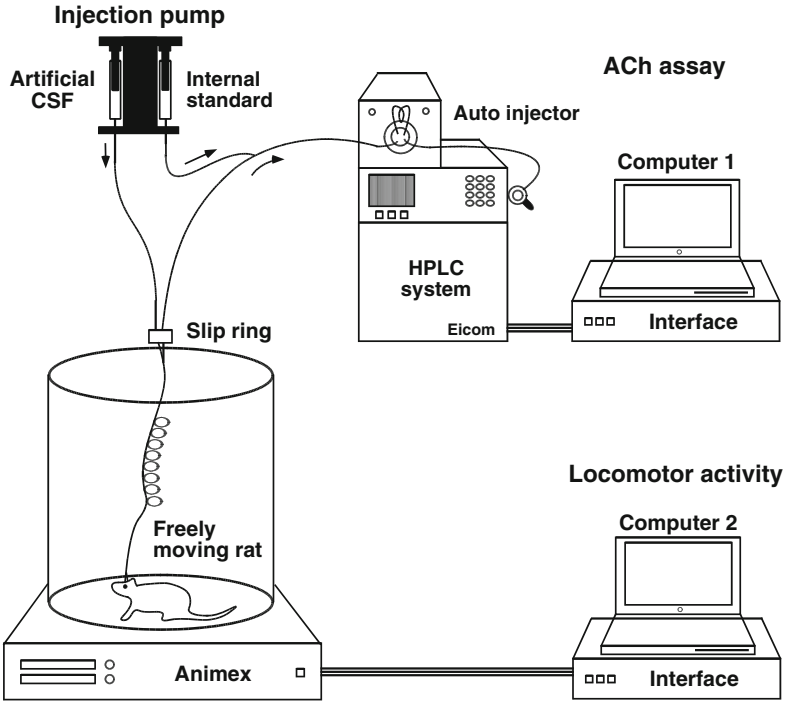

Fig. 2 Experimental setup of in vivo microdialysis system. In order to evaluate the activational effect of sex hormones on ACh release, we simultaneously measured spontaneous locomotor activity in the same subject

inside of the membrane with artificial cerebrospinal fluid, and assayed ACh in dialysates using a high-performance liquid chromatography system. As a result, we were successful in determining an in vivo ACh release profile in selected brain areas in freely moving rats (Fig. 2).

4 ACh Release in the Hippocampus Is Time-Dependent

Using this in vivo measuring system, we showed a temporal 24-h profile of ACh release in the hippocampus. ACh release was episodically observed during the dark phase, but the episodic release was not frequently observed during the light phase (Mitsushima et al. 1998; Masuda et al. 2005). Simultaneous monitoring of spontaneous behavior revealed that the temporal pattern of ACh release is highly correlated with spontaneous movement in freely moving rats (Day et al. 1991; Mizuno et al. 1991; Mitsushima et al. 1996). Since a restriction of exploratory behavior reduces ACh levels and also spatial learning (Mitsushima et al. 1998, 2001), episodic spontaneous behaviors may activate ACh release. In

addition, spontaneous behavior stimulates electrical activity of cholinergic neurons in the basal forebrain (Buzsáki et al. 1988). Moreover, voluntary running enhances neurogenesis, spatial learning, and synaptic plasticity in mice (van Praag et al. 1999). Interestingly, this daily change is quite similar to the daily rhythm in hippocampal mitogen activated protein kinase (MAPK) activity and cAMP: phosphorylated extracellular signal-regulated (ERK) protein, GTP-bound Ras protein, and cAMP in the hippocampus show clear daily changes in male mice (Eckel-Mahan et al. 2008). Although the time-resolution of molecular changes may be low at present, it would be of interest to elucidate the intracellular signaling change with spontaneous behavior in future.

5 Sex Differences in ACh Release

We first reported sex-specific ACh release in the hippocampus in 2003 (Mitsushima et al. 2003a). Gonadally intact male rats consistently show a greater ACh release in the hippocampus compared with diestrous or proestrous female rats, suggesting a sexually dimorphic septo-hippocampal cholinergic system. Moreover, we found that sex-dependent ACh release also shows a time-dependent 24-h profile: ACh release in the hippocampus was relatively similar in the light phase, but consistently lower in female compared with male rats in the dark phase (Masuda et al. 2005). Although ACh release clearly showed a daily rhythm in female rats, females exhibited smaller amplitude of daily change than males. However, it is necessary to rule out the possibility that the sex difference in ACh release reflects the differences in spontaneous locomotor activity levels. By simultaneous monitoring of ACh levels and spontaneous locomotor activity, we revealed a real sex difference in the "ACh release property" (Fig. 3, Mitsushima et al. 2009): males showed higher ACh release than females while displaying similar levels of behavioral activity. Although female rats showed slightly higher overall spontaneous activity than intact male rats, male rats showed higher ACh release than female rats. Simple linear regression analysis was used to evaluate the relationship between ACh levels and spontaneous locomotor activity (Fig. 3). Pearson's correlation coefficient (r) or slope of the best fit line was calculated for each rat, and sex difference was evaluated using ANOVA. We found that the data from intact males had a steep slope of fit line, while the data from females had a gentle slope. These results suggest that sex-specific ACh release is not due to the change in spontaneous behavior, but due to actual differences in the ACh release property in gonadally intact rats (Mitsushima et al. 2009).

To analyze the sex difference in the septo-hippocampal cholinergic neurons, we performed immunocytochemistry. Stereological analysis showed that no sex difference was observed in the number of choline acetyltransferase immunoreactive (ChAT-ir) cells in the medial septum or horizontal limb of diagonal band (Takase et al. 2009). Since the number of septo-hippocampal cholinergic neurons does not appear to be involved in the sex difference in ACh release in the hippocampus, we

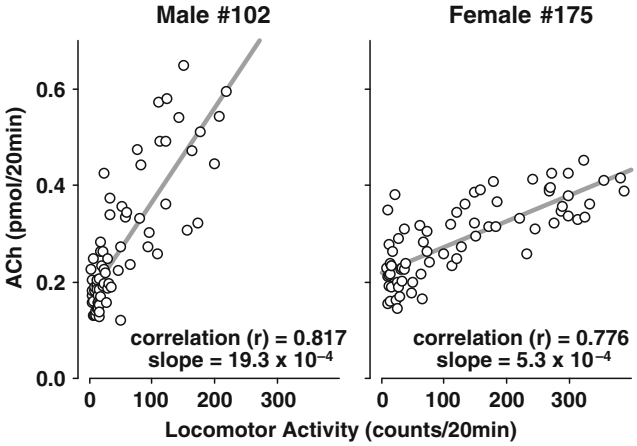

Fig. 3 Sex specific ACh release property in behaving rats. Representative data from a male (#102) and a female (#175) rat were shown. Simple linear regression analysis revealed a sex-specific "ACh release property." Male rats showed higher ACh release than females undergoing similar behavioral activity levels. Although both sexes showed a high correlation, male rats showed a steeper slope than female rats. (see Mitsushima et al. 2009)

hypothesized that sex-specific neural circuits or substance(s) may control the endogenous release.

6 Neural Control of Septo-Hippocampal Cholinergic Neurons

Neurotransmitters may be involved in expression of the sex difference in ACh release. For instance, dopaminergic neurons in the ventral tegmental area (A10) have been shown to control septo-hippocampal cholinergic neurons through the A10-septal dopaminergic pathway in male rats (Swanson 1982; Nilsson et al. 1992; Yanai et al. 1993). A neuroanatomical study suggested that dopamine D_2 receptors rather than D_1 receptors mediate the dopaminergic control of septo-hippocampal cholinergic neurons (Weiner et al. 1991). It has been shown that opiatergic neurons also control septo-hippocampal cholinergic neurons in male rats (Mizuno and Kimura 1996); the injection of naloxone, a μ opioid receptor antagonist, into the medial septum markedly increased ACh release in the hippocampus, while a μ opioid receptor agonist decreased its release (Mizuno and Kimura 1996). In contrast, GABA seems to inhibit septo-hippocampal cholinergic neurons; the injection of muscimol, a GABA receptor agonist, into the medial septum decreased ACh release in the hippocampus, while the injection of bicuculline, a GABA receptor antagonist, increased it (Moor et al. 1998). Although the neural systems are still unknown for female rats, it seems likely that neural control of septo-hippocampal

cholinergic neurons is involved in the expression of sex differences in ACh release. It will be important to investigate these neural systems in female rats in future studies.

7 Circulating Sex Steroids Activate ACh Release

Not only neurotransmitters, but also circulating sex steroids, may regulate cholinergic neurons. In fact, neuroanatomical studies have demonstrated that, in intact male and female rats, a number of dopaminergic neurons in the A10 region have androgen receptor immunoreactivity (Kritzer 1997) and 45–60% of cholinergic neurons in the medial septum have estrogen receptor α immunoreactivity (Miettinen et al. 2002; Mufson et al. 1999). Taken together with the fact that female rats show a greater circulating estrogen concentration than male rats (Shors et al. 2001; Mitsushima et al. 2003b) and male rats show a greater circulating androgen concentration than female rats (Falvo et al. 1974; Rush and Blake 1982), it is possible that cholinergic neurons are affected by sex steroids differently in male and female rats.

The activational effects of sex steroids on cholinergic neurons have been suggested by previous neuroanatomical and neurochemical findings. For example, male gonadectomy decreases the density of cholinergic fibers in the dorsal hippocampus, while testosterone replacement in gonadectomized male rats maintains fiber density (Nakamura et al. 2002). Also, estradiol increases the induction of choline acetyltransferase in the basal forebrain in gonadectomized female rats (Luine et al. 1986; McEwen and Alves 1999). A previous in vitro study demonstrated that estradiol treatment increases both high affinity choline uptake and ACh synthesis in basal forebrain neurons (Pongrac et al. 2004). Furthermore, we recently reported an activational effect of sex steroids on the maintenance of stress-induced ACh release in the dorsal hippocampus in immobilized rats (Mitsushima et al. 2008). These findings suggest the activational effect of sex steroids on ACh release in the dorsal hippocampus, and we presented conclusive evidence of activational effects on dynamic ACh changes in behaving animals. To analyze the precise effects of sex steroids on ACh release, we simultaneously analyzed ACh release and spontaneous locomotor activity to determine the precise effect of sex steroids. Simultaneous analysis revealed that gonadectomy severely impaired ACh release without affecting spontaneous locomotor activity levels. Moreover, the activational effect on ACh release was apparent, especially during the active period, i.e., the dark phase, but not during the rest period, the light phase (Fig. 4 and Mitsushima et al. 2009). Our results provide the first evidence that the sex-specific 24-h profile of ACh release is highly dependent on the presence of sex steroids.

Moreover, we found that after gonadectomy, the positive correlation between ACh release and locomotor activity levels was severely impaired, suggesting that hippocampal function may not always be activated at low sex steroid levels (Mitsushima et al. 2009). This therefore suggests that learning impairment in gonadectomized rats (Gibbs and Pfaff 1992; Daniel et al. 1997; Kritzer et al. 2001;

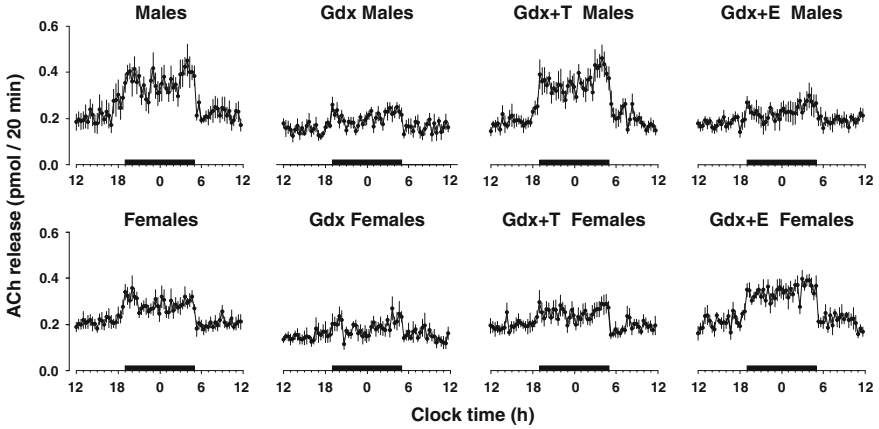

Fig. 4 ACh release in the hippocampus is time-dependent, sex-specific, and hormone-dependent. Experiments were performed 2 weeks after gonadectomy or steroid replacement. Gdx, gonadectomized. +T, testosterone-priming. +E, estradiol-priming. The number of animals was 6–8 in each group. 19–5 h is the dark phase, shown as *black bars* on the x axes. (see Mitsushima et al. 2009)

Markowska and Savonenko 2002; Luine et al. 2003) may be due to insufficient activation of hippocampus at the appropriate time. Because the replacement of sex-specific steroids restored the high positive correlation between ACh release and activity levels, the correlation appears to depend on the presence of sex steroids. These results suggest that circulating sex steroids strengthen the coupling between spontaneous behavior and ACh release (Mitsushima et al. 2009).

8 Sexual Differentiation Produces the Sex-Specific Activational Effect

The activational effect of sex steroids was sex-specific (Fig. 4). Testosterone replacement in gonadectomized female rats failed to increase ACh release to levels seen in gonadectomized testosterone-primed male rats. Similarly, estradiol replacement was unable to restore ACh release in gonadectomized male rats. Moreover, estradiol consistently increases N-methyl-D-aspartate receptor binding and spine density in the CA1 area of gonadectomized female rats, although the treatment fails to increase these same parameters in gonadectomized male rats (Romeo et al. 2005; Parducz et al. 2006). These results suggest that sex-specific steroids are important for maintaining hippocampal function. Based on our data, we hypothesized that the action of sex-specific steroids is due to neonatal sexual differentiation rather than the activational effects of sex steroids in adult rats. Moreover, in the latest study, we found that neonatal androgenization in females increased ACh release to resemble that of normal males without affecting spontaneous activity levels (Mitsushima

et al. 2009). These results indicate an organizational effect on sex-specific ACh release in behaving rats, and support currently accepted theories of sexual differentiation.

Because testosterone can be aromatized to estradiol in the forebrain, neonatal sex steroids activate both estrogen and androgen receptors (McEwen 1981). In our study, both testosterone and estradiol treatment in neonatal female pups masculinized ACh release profile in adults, suggesting an estrogen receptor-mediated masculinization of septo-hippocampal cholinergic systems (Mitsushima et al. 2009). These results are consistent with the previous finding that testosterone or estradiol treatment in neonatal female pups improves their adult spatial performance, whereas neonatal gonadectomy in male pups impairs the performance (Williams and Meck 1991). In contrast, dihydrotestosterone treatment failed to masculinize the ACh release profile. Although dihydrotestosterone has been classically considered as a prototypical androgen receptor agonist, a metabolite of dihydrotestosterone, 3β-diol, has a higher affinity for estrogen receptor β (Lund et al. 2006). Therefore, dihydrotestosterone and its metabolites may stimulate both androgen receptor and estrogen receptor β, whereas estradiol stimulates estrogen receptors α and β. Considering the action of sex steroids and their metabolites, estrogen receptor α may mediate the organizational effect on the septo-hippocampal cholinergic system.

9 Interaction with Environmental Conditions

Various environmental conditions may interact with the activational effects of sex steroids. First, we reported an interaction between stress and sex steroids. Although sex steroids did not show activational effects on baseline levels of ACh release, sex steroids clearly activated the immobility stress-induced ACh release response. In addition, we found that the contributing sex hormone effect to maintain the ACh release response was sex-specific: testosterone enhanced the ACh release response in male rats, while estradiol maintained the response in females (Mitsushima et al. 2008). Second, we reported an interaction between the light/dark cycle and sex steroids. Although sex steroids slightly enhanced ACh release during the light phase, the activational effects were much stronger during the dark phase (Fig. 4). Considering the fact that the time-dependent activational effect was also sex-specific and hormone-dependent, environmental conditions seem to have complicated interactions with sex steroids (Mitsushima et al. 2009).

Some other environmental effects may affect the basal forebrain cholinergic system. Environmental conditions, such as complex or restricted (Brown 1968; Smith 1972), enriched or impoverished (Greenough et al. 1972), social or isolated conditions (Hymovitch 1952; Juraska et al. 1984; Seymoure et al. 1996), seem to affect spatial learning ability in a sex-specific manner. For example, male rats exhibited superior performance in learning maze tests compared with female rats if they were housed socially (Einon 1980). But if they were housed in isolation,

female rats exhibited a performance superior to that of male rats (Einon 1980). Although few studies were performed on the relationship between the sex-specific environmental effects and ACh release in the brain, we have reported that 4-day housing in a small cage attenuates the ACh release in the hippocampus in male rats (Mitsushima et al. 1998), but not in female rats (Masuda et al. 2005). Taken together, these results suggest that housing conditions contribute to the sex difference in ACh release and spatial learning ability.

Feeding conditions after weaning also affect spatial learning ability. If fed pelleted diet (i.e., standard laboratory diet), male rats show performance superior to that of female rats (Beatty 1984; Williams and Meck 1991). But when fed powdered diet, female rats, but not male rats, showed improved performance (Endo et al. 1994; Takase et al. 2005a). In our study, it was found that feeding with powdered diet after weaning increased ACh release in the hippocampus in female rats, but not in male rats (Takase et al. 2005b). A 24-h ACh release in female rats fed powdered diet was as high as that in male rats fed either powdered or pelleted diet, showing no sex difference. Since feeding with powdered diet improved spatial learning ability in female rats (Endo et al. 1994), the increase in the ACh release in the hippocampus in female rats fed powdered diet may partly contribute to this effect. Our findings provide evidence that environmental conditions such as housing or feeding may play a role in sex-specific hippocampal function.

10 A Possible Treatment Strategy for Alzheimer's Disease

Activational effects of sex steroids are very important in humans, since circulating sex steroid levels decline with age. A reduction in ACh synthesis is known as a common feature of Alzheimer's disease (Coyle et al. 1983), afflicting more than 18 million people worldwide (Ferri et al. 2005; Mount and Downtown 2006). The disease is the most common form of dementia (Cummings 2004) and is frequently accompanied by insomnia, poor concentration, and day/night confusion (McCurry et al. 2004; Starkstein et al. 2005). The centrally active acetylcholinesterase inhibitor (donepezil) is effective in not only mild, but also moderate to severe cases (Petersen et al. 2005; Winblad et al. 2006), proving the importance of endogenous ACh in humans. In addition, women are twice as likely to develop the disease (Swaab and Hofman 1995), and estradiol seems to play a protective role (Zandi et al. 2002; Norbury et al. 2007). A recent study using single photon emission tomography showed that estrogen replacement therapy in healthy postmenopausal women increases muscarinic M_1/M_4 receptor binding in the hippocampus (Norbury et al. 2007). Conversely in men, testosterone but not estradiol seems to play a protective role (Moffat et al. 2004; Rosario et al. 2004) and testosterone supplementation clearly improved hippocampal-dependent learning deficits in men with Alzheimer's disease (Cherrier et al. 2005). These results suggest a sex-specific activational effect of gonadal steroids on the cholinergic system in humans. Thus, there are many similarities between the rat model and the human studies, supporting

the idea that gonadal steroid replacement therapy or an increase in bioavailability is beneficial when there is a subthreshold level of the hormone. Based on the neonatal sexual differentiation of the septo-hippocampal cholinergic system, we may have to search for sex-specific clinical strategies for Alzheimer's disease.

11 Conclusions

Gonadally intact male rats consistently show a greater ACh release in the hippocampus compared with diestrous or proestrous female rats. The activational effects of sex steroids are important for sex-specific ACh release in the hippocampus, since impaired ACh release in gonadectomized rats does not show sex-specific effects. Neonatal treatment with either testosterone or estradiol clearly increased ACh release in female rats, suggesting neonatal sex differentiation of septo-hippocampal cholinergic systems. Moreover, environmental effects on the basal forebrain cholinergic system seem to be sex-specific; housing in a small cage attenuated ACh release in male rats only, while feeding with powdered diet after sexual maturation increases ACh release in female rats only. These results indicate that: (1) sex-specific circulating sex steroids are necessary for sex-specific ACh release, (2) neonatal activation of estrogen receptors is sufficient to mediate masculinization of the septo-hippocampal cholinergic system, and (3) sex-specific effects of environmental conditions may suggest an interaction with the effect of sex hormones.

Understanding the importance of gonadal steroids and the sex-specific effects in cognitive disorders such as Alzheimer's disease is essential for real improvements in therapy.

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Females Are More Vulnerable to Drug Abuse than Males: Evidence from Preclinical Studies and the Role of Ovarian Hormones

Justin J. Anker and Marilyn E. Carroll

Contents

Abstract Human and animal research indicates the presence of sex differences in drug abuse. These data suggest that females, compared to males, are more vulnerable to key phases of the addiction process that mark transitions in drug use such as initiation, drug bingeing, and relapse. Recent data indicate that the female gonadal hormone estrogen may facilitate drug abuse in women. For example, phases of the menstrual cycle when estrogen levels are high are associated with enhanced positive subjective measures following cocaine and amphetamine administration in women. Furthermore, in animal research, the administration of estrogen increases drug taking and facilitates the acquisition, escalation, and reinstatement of cocaine-seeking behavior. Neurobiological data suggest that estrogen may

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facilitate drug taking by interacting with reward- and stress-related systems. This chapter discusses sex differences in and hormonal effects on drug-seeking behaviors in animal models of drug abuse. The neurobiological basis of these differences and effects are also discussed.

Keywords Drug abuse · Estrogen · Progesterone · Rats · Sex differences · Sex hormones

1 Introduction

Historically, drug abuse has been considered a male disease. Research from several areas including epidemiology, behavioral pharmacology, and neuroscience has taken a male-centric approach when studying factors and/or treatments that influence drug abuse. This approach has led to neglect of factors underlying drug abuse in women such as ovarian hormones. In fact, over the centuries, the predominant sex that abused drugs has varied from female to male, depending on cultural conditions (Kornetsky 2007). However, in recent years, epidemiological research has shown that females are catching up and exceeding males in their drug use, particularly among younger populations. Thus, an important direction for current research is to acknowledge that sex is a vulnerability factor in drug abuse and to study the neurobiological basis for this trend and its implications for drug abuse treatment (Ashley et al. 2003; Marsh et al. 2004).

Clinical and preclinical research indicates that females, compared to males, exhibit greater vulnerability toward drug abuse at stages of the addiction process that mark transitions in drug use. These stages include drug initiation, bingeing, withdrawal, and relapse and may be modeled in animals using acquisition, escalation, withdrawal/extinction, and reinstatement procedures, (Carroll et al. 2009a). Clinical reports indicate that women are more likely than men to initiate drug use at an earlier age (Chen and Kandel 2002), engage in binge-like patterns of drug intake (Becker and Hu 2008; Brady and Randall 1999; Lynch et al. 2002; Mann et al. 2005; Randall et al. 1999), report greater difficulty in quitting (Becker and Hu 2008; Carpenter et al. 2006; Lynch et al. 2002), exhibit greater drug craving (Robbins et al. 1999), relapse (Ignjatova and Raleva 2009), and resume higher levels of drug use following relapse (Gallop et al. 2007). One area in which males exceed females in the phases of drug abuse is during withdrawal where males experience more severe withdrawal effects than females (Carroll et al. 2009b; Perry et al. 2008). Thus, elevated drug use in females may be due not only to their greater sensitivity to rewarding effects but also to their resilience to the negative effects of drugs. This differential sensitivity to the rewarding and aversive aspects of drug use has parallels in other addiction-prone and -resistant phenotypes (Carroll et al. 2009a), and it is an emerging area of research that should yield interesting developments.

Animal models of drug abuse add further support to enhanced female vulnerability across most stages of the addiction process. Female rats acquire drug self-administration at a faster rate, they exhibit greater binge-like patterns of drug intake, and they are more vulnerable to relapse of drug-seeking behavior (for reviews, see Carroll and Anker 2009; Carroll et al. 2009a; Lynch et al. 2002). The animal data suggest that females may be more vulnerable than males due to an underlying biological predisposition related to ovarian hormones or developmental/organizational differences in male and female neurobiology (Becker and Hu 2008). As a consequence, it is important to identify biological vulnerability factors that contribute to the onset and progression of drug addiction in women.

A growing number of findings suggest that the biological basis for increased drug abuse vulnerability in women may be attributed to female gonadal hormones (Becker and Hu 2008; Carroll et al. 2004; Festa and Quinones-Jenab 2004; Lynch et al. 2002; Terner and de Wit 2006). More specifically, enhanced drug-seeking and subjective effects in women are associated with higher levels of endogenous estrogen (EST) (Evans 2007; Terner and de Wit 2006). Further work supports this and has demonstrated that endogenous or exogenous EST (e.g., estradiol benzoate, 17β-estradiol) facilitates the acquisition, escalation, and reinstatement of cocaine-seeking behavior in female rats (for a review, see Carroll and Anker 2010). Preclinical work further suggests that EST's potentiating effects on drug-related responses may involve activation of the EST-receptor subtype β (ER-β), whereas ER-α had little influence on drug seeking (Larson and Carroll 2007). In contrast to the potentiating effects of EST, progesterone (PROG), another female gonadal hormone, attenuates responses to drugs of abuse in both humans and animals.

This chapter emphasizes sex and hormonal influences across several phases of the human drug abuse process that are represented by animal models. Particular attention is given to the effects of EST on drug-related responses; however, as PROG often opposes EST's behavioral effects, its influence on drug-seeking behavior is also important to consider. The final section of this chapter discusses possible neurobiological mechanisms underlying sex differences and EST's effects on addiction-related behaviors. EST's interactions within the mesolimbic dopamine (DA) system, the hypothalamic-pituitary-adrenal (HPA) axis, and the involvement of ER-β receptors are considered. Since a majority of research on sex differences and hormonal influences on drug abuse involves psychomotor stimulants, this drug class is the primary focus.

2 Menstrual Cycle and Hormonal Effects on Responses to Drugs of Abuse: Clinical Evidence

Natural fluctuations of hormones during the menstrual cycle correspond to differences in the physiological and subjective effects of some stimulant drugs

(Evans 2007; Terner and de Wit 2006). Cardiovascular and/or positive subjective responses to cocaine (Evans and Foltin 2006; Evans et al. 2002; Sofuoglu et al. 1999) and amphetamine (Justice and de Wit 1999, 2000; White et al. 2002), but not nicotine (Terner and de Wit 2006), were enhanced during the EST-dominant follicular phase of the menstrual cycle compared with the luteal phase (when EST levels are low) in women. These results were also extended to measures of stress reactivity and craving elicited by cocaine-related stimuli in cocaine-dependent women (Sinha et al. 2007). In this study, women in the follicular phase showed higher systolic/diastolic blood pressure measures, and they scored higher on self-reported measures of anxiety and drug craving following presentations of stressful or drug-related stimuli than women during the midluteal phase (Sinha et al. 2007). The finding with stress-induced craving is especially important given that clinical and preclinical reports implicate stress as a primary factor in drug bingeing and relapse (Covington et al. 2005; Sinha 2008), and stress is associated with heightened drug abuse vulnerability in women (Fox and Sinha 2009). Taken together, these results indicated that the phase of the menstrual cycle in which EST levels were highest was associated with high positive affective responses to cocaine and enhanced cue- and stress-induced cocaine craving. Thus, EST may operate as a vulnerability factor that facilitates the positive and aversive aspects of cocaine abuse in women. Others have found no differences in the physiological and subjective responses to cocaine during the female menstrual cycle (Lukas et al. 1996; Mendelson et al. 1999). However, methodological differences related to the dose and route of cocaine administration may account for the discrepancy in results.

Compared to results with stimulants, behavioral/subjective responses to other drugs of abuse do not consistently vary with the phase of the menstrual cycle in humans. Subjective measures were insensitive to menstrual cycle effects following alcohol (Freitag and Adesso 1993; Hay et al. 1984; Holdstock and de Wit 2000; Nyberg et al. 2004; Sutker et al. 1987), nicotine (Allen et al. 1999, 2004; Pomerleau et al. 1992, 2000; Snively et al. 2000), marijuana (Lex et al. 1984), and opioid (Gear et al. 1996) administration in women.

In contrast to the results with EST, PROG has an opposite effect on subjective measures following drug administration (for review, see Evans 2007). Women treated with PROG showed a decrease in the positive-subjective effects of smoked (Evans and Foltin 2006; Sofuoglu et al. 2002) and iv (Sofuoglu et al. 2004) cocaine compared with placebo-treated controls. In addition, high circulating plasma levels of PROG were associated with decreased craving following a stress- or drug-related cue in cocaine-dependent women (Sinha et al. 2007).

Overall, results from clinical studies indicate that the female gonadal hormone, EST, may be associated with sex differences in cocaine abuse, as phases of the menstrual cycle associated with heightened EST corresponded to increases in positive-subjective measures following cocaine. In contrast, PROG had an attenuating effect on these measures and suppressed stress- and cue-induced drug craving.

3 Sex and Ovarian Hormones Influence Drug Seeking and Drug Taking: Preclinical Evidence

Preclinical studies corroborate clinical findings and confirm the importance of sex differences and hormonal influences during key phases of the addiction process as they are modeled in animals. In animal research, the self-administration paradigm is considered a valid model of human drug addiction (Panlilio et al. 2007), as subjects have control over their self-administration of the drug. Animal models of drug self-administration allow a controlled longitudinal approach to the study of factors that predict drug abuse in addition to potential treatments during critical transition phases of the drug abuse process (Carroll et al. 2009a). Phases to be discussed are acquisition (initiation) of drug use, maintenance of steady drug intake, bingeing or escalation of drug intake, extinction (withdrawal), and reinstatement (relapse). The following section discusses sex differences and the effects of EST with regard to these important transition phases of drug addiction. Results from these studies are summarized in Table 1.

3.1 Acquisition

In animal research, acquisition or the initiation of drug self-administration is measured using several techniques that primarily involve automatic (e.g., autoshaping) or experimenter-administered priming infusions of drug prior to, or at the beginning of, each self-administration session. Animals achieve acquisition criteria once they earn a predefined number of self-administered drug infusions.

3.1.1 Sex Differences

Similar to clinical findings (Chen and Kandel 2002), females acquired drug self-administration faster than male rats across a wide range of drugs including cannabinoids (Fattore et al. 2007), cocaine (Jackson et al. 2006; Lynch 2008; Lynch and Carroll 1999b), methamphetamine (Roth and Carroll 2004a), nicotine (Chaudhri et al. 2005), and heroin (Carroll et al. 2002; Lynch and Carroll 1999b). Research with monkeys also confirmed that females acquired PCP self-administration more successfully than males, with 100% of the females acquiring compared to only 36.4% of the males (Carroll et al. 2000). Together, these results indicated that females had increased vulnerability to initiate drug use compared with males.

3.1.2 Hormonal Influences

Preclinical work implicates EST in sex differences in the acquisition of drug abuse. Two methods are involved in investigating the contributions of EST and PROG in

Table 1 Summary of the sex differences and estrogen effects on behavioral responses to drugs of abuse across phases of the drug abuse process: animal models

Independent variable	Dependent measure	Drug	Finding	Reference
Sex differences (monkey)	Acquisition	PCP	F > M	Carroll et al. (2000)
	Escalation	PCP	F > M	Carroll et al. (2005)
Sex differences (rat)	Acquisition	Cannabinoids	F > M	Fattore et al. (2007)
		Cocaine	F > M	Lynch and Carroll (1999b), Lynch (2008), Jackson et al. (2006)
		Methamphetamine	F > M	Roth and Carroll (2004a)
		Nicotine	F > M	Chaudhri et al. (2005)
		Heroin	F > M	Lynch and Carroll (1999b), Carroll et al. (2002)
	Escalation	Cocaine	F > M	Lynch et al. (2000), Lynch and Taylor (2004, 2005), Roth and Carroll (2004b)
	Extinction	Cocaine	F > M	Lynch and Carroll (2000), Kippin et al. (2005), Lynch et al. (2005), Kerstetter et al. (2008), Perry et al. (2008)
	Cocaine-primed reinstatement	Cocaine	F > M	Lynch and Carroll (2000), Kerstetter et al. (2008), Anker et al. (2009)
	Stress-induced reinstatement	Cocaine	F > M	Anker and Carroll (2010)
	Cue-induced reinstatement	Cocaine	M < F	Fuchs et al. (2005)
Systemic estrogen administration	Acquisition	Cocaine	OVX-E > OVX-V	Lynch et al. (2001), Jackson et al. (2006)
			OVX-E > OVX-E+P	Jackson et al. (2006)
		Heroin	OVX-E > OVX-V	Roth et al. (2002)
	Escalation	Cocaine	OVX-E > OVX-V	Lynch and Taylor (2005), Larson et al. (2007)
			OVX-E > OVX-E+P	Larson et al. (2007)
	Cocaine-primed reinstatement	Cocaine	OVX-E > OVX-V	Larson et al. (2005), Anker et al. (2007), Larson and Carroll (2007)
			OVX-E > OVX-E+P	Anker et al. (2007)
			OVX-ERbeta > OVX-ERalpha, OVX-V	Larson and Carroll (2007)

F Female, *M* Male, *E* Estrogen, *V* vehicle, *P* PROG, OVX ovariectomy

animal models of drug abuse. The first involves comparing drug-seeking behavior across different phases of the ovarian hormone cycle, and the second involves depleting naturally occurring levels of hormones through ovariectomy (OVX), administering EST and/or PROG, measuring the addiction-related response, and comparing this response to gonadally intact sham (SH)-operated controls.

The acquisition rates for iv cocaine self-administration were reduced in OVX female rats compared with SH-operated females (Jackson et al. 2006), whereas the administration of EST in OVX females facilitated the acquisition of cocaine (Jackson et al. 2006; Lynch et al. 2001) and heroin (Roth et al. 2002) self-administration. The injection of the EST receptor antagonist, tamoxifen, blocked EST's facilitation of cocaine acquisition in OVX female rats (Lynch et al. 2001). In contrast to EST, PROG had an opposite effect on the acquisition of drug self-administration. For example, Jackson et al. (2006) showed that PROG treatment blocked the effects of EST on the acquisition of cocaine self-administration in OVX female rats. Together, the results suggest that EST and PROG have opposite roles during the initiation of drug self-administration in females.

3.2 Escalation

The transition from steady to dysregulated drug consumption characterizes the escalation phase of the drug abuse process (Ahmed and Koob 1998, 1999; Lynch and Carroll 1999a). In humans, escalation represents out-of-control drug bingeing that is linked to overdose and death (Kalivas and Volkow 2005). Females, compared to males, are more susceptible to binge-like drug intake (Brady and Randall 1999; Mann et al. 2005; Randall et al. 1999). Thus, it is important to use animal models to identify factors that contribute to the development of this critical aspect of drug addiction in women. Animal models offer a means to examine sex differences and hormonal influences on binge-like drug intake.

3.2.1 Sex Differences

Drug bingeing is modeled in animals using an extended-access procedure. In these studies, long access (LgA) to a self-administered drug (e.g., 6 h) results in increased drug intake over subsequent days (Ahmed and Koob 1999). In a study by Roth and Carroll (2004b), female rats escalated cocaine intake to a greater extent than males during LgA. Furthermore, females responded significantly more for iv infusions of cocaine at lower doses (Roth and Carroll 2004b). Similar results were reported with rhesus monkeys self-administering PCP (Carroll et al. 2005) providing cross-species evidence for sex differences. Females and males did not differ in PCP intake under a short-access condition (ShA) (3 h); however, females exceeded males in mg/kg PCP intake when access was extended to 6 h. Dose–response functions under a ShA progressive-ratio (PR) schedule before and after the LgA

condition indicated both groups experienced a rightward shift in their dose–response curves following LgA and that females (vs. males) consumed more PCP across several concentrations under the post-LgA PR condition (Carroll et al. 2005).

Escalation of drug intake has also been modeled in animals using a dose self-selection procedure in which animals achieve a preferred dose of drug by responding on two levers that respectively increase or decrease the infusion duration (Lynch and Carroll 2001; Lynch et al. 1998). Female rats exhibited greater dysregulation of drug intake compared with males as determined by a lower correlation between the interdose interval and the preceding dose size, and females responded on the dose-increasing lever more than males (Lynch et al. 2000).

Another method for assessing excessive drug intake in animal models is the discrete-trials procedure that allows two to four 10-min trials/h during self-administration. In a study by Lynch and Taylor (2004), male and female rats self-administered similar amounts of cocaine under ShA FR 1 and PR schedules. However, when rats were subsequently placed on the discrete-trials procedure for 7 days, females self-administered significantly more cocaine than males and showed greater disruption in diurnal self-administration patterns (Lynch and Taylor 2005). When performance under the PR schedule was reassessed 10 days following the discrete-trials procedure, females surpassed males in cocaine infusions.

3.2.2 Hormonal Influences

In a study by Larson et al. (2007), the effects of EST and PROG were examined on cocaine self-administration under ShA and LgA conditions. Five groups were compared: OVX-VEH, OVX-EST, OVX-EST+PROG, SH-VEH, and SH-PROG. Prior to LgA, all groups exhibited similar levels of ShA cocaine intake, a finding consistent with previous studies (Cain et al. 2004; Larson et al. 2005; Lynch and Carroll 2000; Roth and Carroll 2004b). However, when access was extended to 6 h/day (LgA), groups SH-VEH, OVX-EST, and OVX-VEH escalated cocaine intake, whereas the PROG-treated groups (SH-PROG, OVX-EST+PROG) did not. Furthermore, OVX EST-treated rats escalated their drug intake more rapidly and self-administered more cocaine during LgA than OVX-VEH rats. Thus, exogenously administered EST facilitated the escalation of cocaine intake, whereas PROG attenuated it.

Similar results have been reported with the 24 h/day discrete-trials procedure. Lynch and Taylor (2005) demonstrated that OVX rats treated with VEH earned fewer cocaine infusions under the discrete-trials procedure when compared with SH-operated controls, and EST enhanced drug intake in OVX female rats relative to VEH-treated controls to levels of the SH-operated rats (Lynch and Taylor 2005). Taken together, these results suggest that EST is involved in enhanced escalation of drug taking in females relative to males.

3.3 Extinction/Reinstatement (Relapse)

Relapse is one of the most difficult aspects of drug abuse to treat due to the craving and other withdrawal effects that result in its high rate of recurrence (McKay and Weiss 2001). As previously mentioned, women are especially vulnerable to drug abuse relapse following a period of abstinence (Ignjatova and Raleva 2009), and once relapse occurs, women are prone to consume excessive amounts of drug (Gallop et al. 2007). Relapse is modeled in animals using the reinstatement procedure. Typically, animals are allowed to self-administer a drug for several days (usually 10–14 days). Drug solutions are then removed or replaced with saline, and animals subsequently extinguish their responding for the drug over the next 2–3 weeks. Subsequently, a priming stimulus consisting of the drug, a drug-associated cue, or a physical (e.g., shock) or chemical (e.g., yohimbine) stressor is introduced. Subsequent responding on the device previously associated with the drug delivery following one or more of the priming stimuli is considered a measure of reinstatement and a predictor of relapse in humans (Katz and Higgins 2003; Shaham et al. 2003). Results from animal reinstatement studies confirm female vulnerability during this critical stage of the drug abuse process and implicate EST in the facilitation of these measures. The following section discusses these findings.

3.3.1 Sex Differences

Several studies have demonstrated sex differences in the extinction and reinstatement of drug-seeking behavior. During extinction, female (vs. male) rats show greater resistance to extinguishing responding that was previously maintained by iv cocaine infusions (Anker and Carroll 2010; Kerstetter et al. 2008; Kippin et al. 2005; Lynch and Carroll 2000; Lynch et al. 2005; Perry et al. 2008). Elevated responding in female rats relative to males extends to the reinstatement phase as well; however, this depends on the type of reinstatement stimulus used. Female (vs. male) rats responded more on a lever previously associated with cocaine self-administration than male rats following a priming injection of cocaine (Anker et al. 2009; Kerstetter et al. 2008; Lynch and Carroll 2000) and after 1, 14, 60, and 180 days of cocaine withdrawal (Kerstetter et al. 2008). In another study, Anker and Carroll (2010) showed that females reinstated significantly more than males following an injection of the pharmacological stressor, yohimbine. This is especially pertinent, as previous clinical studies indicate that female cocaine addicts are more vulnerable to stress-induced relapse than male cocaine addicts (Fox and Sinha 2009).

3.3.2 Hormonal Influences

During the estrus phase, female rats were more resistant to extinction of lever pressing previously reinforced with cocaine than during any other phases of the rat

estrous cycle (Feltenstein and See 2007; Kerstetter et al. 2008). Furthermore, systemic injection of EST enhanced the cocaine-primed reinstatement responding in OVX female rats relative to OVX rats treated with VEH (Anker et al. 2007; Larson and Carroll 2007; Larson et al. 2005). Larson and Carroll (2007) examined the effects of ER-α and ER-β on cocaine-seeking behavior under a reinstatement procedure. Following extinction of lever pressing, OVX rats received acute systemic injections of EST (ER-α and ER-β agonist), the ER-α agonist propylpyrazole-triol (PPT), or the ER-β agonist diarylpropionitrile (DPN). They were then tested on cocaine-primed reinstatement of cocaine seeking. The results indicated that EST- and DPN-treated OVX rats reinstated significantly more than OVX rats treated with VEH, while there were no differences in reinstatement responding in OVX rats treated with PPT compared with those treated with VEH. Thus, EST may facilitate reinstatement responding via activation of ER-β. In contrast to the results with EST, PROG and its metabolite, allopregnanolone (ALLO), have opposite effects on animal models of relapse. Increases in plasma PROG levels in freely cycling female rats were associated with decreased reinstatement responding following a cocaine priming injection (Feltenstein and See 2007). In addition, systemic injections of PROG in SH- and EST-treated OVX female rats attenuated reinstatement responding relative to SH rats treated with VEH and OVX rats treated with EST alone (Anker et al. 2007). The suppression of reinstatement responding by PROG may be attributed to its metabolism into ALLO. In a follow-up study, coadministering finasteride, a 5-α reductase inhibitor that prevents the conversion of PROG into ALLO, blocked PROG's attenuating effects on cocaine-primed reinstatement (Anker et al. 2009). Taken together, these results indicate that female gonadal hormones are involved in susceptibility toward (EST) and protection against (PROG) relapse of cocaine seeking in females.

4 Neurobiological Basis of Sex Differences and EST Effects in Drug Seeking

Drugs interact with motivational systems that regulate survival behaviors, and drug and nondrug stimuli activate common neurobiological systems (Spanagel and Weiss 1999; Wise 1996). General reward-mediated responding involves the interaction of several neuronal systems within the ventral and midbrain areas of the brain that contain the nucleus accumbens (NA) and the ventral tegmental area, collectively referred to as the mesolimbic reward pathway. This section discusses male/female brain dimorphism and the interaction between female sex hormones, and neurotransmitter systems within this motivational pathway, with respect to drug abuse. Most of this research has centered on stimulants, and consequently this drug class will be the focus.

4.1 Brain Dimorphism and Sex Differences in Drug Addiction

Sexual dimorphism in areas of the brain involved in motivation and/or hormone-DA system interactions may play a key role in sex differences in drug abuse. These topics have been reviewed elsewhere (Becker 2009) and are briefly covered here. Masculinization of the brain occurs early during maturation (perinatal period) and is largely attributed to the gonadal hormone testosterone (Becker 2009; McCarthy et al. 1997), while feminization occurs in the absence of testosterone. Gonadectomy during this early period of brain sexualization decreased amphetamine-induced DA increases that occurred during adulthood in female rats, while it had no effect on adult males (Becker and Ramirez 1981a). Thus, it is hypothesized that sexual differentiation of key components of the DA system during periadolescence may later sensitize rats to the facilitating effects of EST and contribute to the sex differences in the reinforcing effects of stimulants (Becker 2009).

Morphological differences in areas of the brain that regulate cocaine craving are also observed between sexes in adult humans. For example, men and women differ in the relative size of mesolimbic and mesocortical structures that are implicated in responses to drugs of abuse such as the cerebral cortex (Rabinowicz et al. 1999), medial amygdala (Mizukami et al. 1983), and the hippocampus (Fattore et al. 2008; Filipek et al. 1994).

4.2 Role of DA

4.2.1 Sex Differences

Several lines of research indicate that sex differences and the influence of EST affect neurotransmitter systems that operate in the mesolimbic reward pathway to regulate the abuse-related effects of stimulants. In the striatum, there are clear sex differences in baseline DA tone and activation following exposure to drugs of abuse. Striatal D_1 DA receptors decrease while D_2 receptors increase cocaine-seeking behavior (Becker and Hu 2008; Self et al. 1996). Interestingly, there are approximately 10% more striatal D_1 DA receptors in male rats compared to females (Andersen et al. 1997), which may explain why females outperform males on several measures of cocaine seeking. However, there are reportedly no sex differences in D_2 receptor densities in striatal regions in humans (Farde et al. 1995; Munro et al. 2006). There are also sex differences in extracellular striatal DA concentrations. For example, basal (Castner et al. 1993) and K^+-stimulated (Walker et al. 2000) DA concentrations are greater in female rats compared to males, a finding that may be due to differential affinity for the DA transporter in presynaptic terminals (Walker et al. 2000, 2006). Protein kinase A (PKA) signaling has been shown to alter DA transmission within the mesolimbic reward pathway and is implicated in drug abuse (Nairn et al. 2004; Nestler 2005). In a study by Lynch

et al. (2007), females exhibited higher levels of PKA-mediated phosphorylization of DARPP-32 (DA- and cyclic AMP-regulated phosphoprotein) in the striatum and NA (Nazarian et al. 2009; Zhou et al. 2009), while Nazarian et al. (2009) reported similar results with PKA protein levels in the NA.

Administration of stimulants enhances sex differences in dopaminergic activation in the mesolimbic pathway, and this may also lead to subsequent sex differences in drug reinforcement. For example, females exhibited increased striatal DA following amphetamine than males (Becker and Cha 1989; Becker and Ramirez 1981b), and they were more sensitive to the facilitating effects of cocaine on electrically stimulated DA release than males (Walker et al. 2006). Males and females also differed in activation of the DARPP-32 pathway in the NA following cocaine administration (females < males) (Lynch et al. 2007; Zhou et al. 2009). Striatal DA levels were greater in females than males following the administration of other drugs of abuse. For example, using in vivo microdialysis, Blanchard and Glick (1995) demonstrated that mesolimbic DA levels in the NA were greater in female rats following administration of low-to-moderate doses of alcohol, and female rats consumed more alcohol at these doses than male rats (Blanchard and Glick 1995; Blanchard et al. 1993). Females also exhibited an increased number of DA transporters in the NA following repeated injections of intravenous nicotine (Harrod et al. 2004).

4.2.2 Estrous Cycle and EST

Sex differences in the activation of the mesolimbic DA system have been attributed to circulating hormones. Several studies have demonstrated that EST treatment enhances striatal DA release (Becker 1990a,b, 1999; Becker and Ramirez 1981a; Dazzi et al. 2007; McEwen and Alves 1999; Zhang et al. 2008) and induces conditioned place preference when injected in large doses (Frye and Rhodes 2006) in OVX rats relative to VEH-treated controls. Striatal DA levels are also significantly higher in gonadally intact females compared to OVX female rats (Becker and Beer 1986; Becker et al. 1984; Becker and Ramirez 1981a), suggesting that the absence of EST may decrease DA levels. This may explain why a lack of EST, due to natural fluctuations or pharmacological and/or surgical manipulation (Anker et al. 2007; Larson and Carroll 2007; Larson et al. 2005, 2007; Lynch et al. 2001), leads to attenuated cocaine seeking.

Ligand-bound EST receptors regulate the transcription of proteins involved in the DA system (Jones and Miller 2008). Indeed, D_2 receptor densities in the striatum and other areas of the brain implicated in addiction vary across the estrous cycle in rats. They are greater following natural elevations of EST or following systemic EST administration (Bazzett and Becker 1994; Czoty et al. 2009; Di Paolo et al. 1988; Pazos et al. 1985; Zhou et al. 2002) and decrease significantly within 2 weeks following OVX (Le Saux et al. 2006). In contrast, in one study using positron emission tomography, D2 receptor concentrations were significantly lower

during the EST-dominant follicular compared to the luteal phase of the menstrual cycle.

There is also evidence indicating that intracellular DA activity changes across the estrous cycle in female rats, and this may also contribute to cycle-dependent alterations in responses to drugs of abuse. Weiner et al. (2009) demonstrated that phosphorylated DARPP-32 levels in female rats were significantly lower during the estrus phase compared to all other phases of the estrous cycle. This result was explained as a consequence of heightened DA levels in the NA during the estrus phase, and lower DARPP-32 levels reflected a compensatory mechanism to stabilize excessive DA concentrations (Weiner et al. 2009).

Research also indicates hormone cycle mediation of dopaminergic responses to stimulants. For example, amphetamine-stimulated DA release in striatal tissue was increased during the estrus phase of the estrous cycle as determined using in vitro infusion (Becker and Ramirez 1981b), microdialysis (Becker and Cha 1989), and voltammetry (Becker 1990b). Several studies also implicate EST in modulating stimulant-induced dopaminergic activity. For example, EST treatment in OVX rats promoted DA neuronal sensitivity to cocaine, while DA neurons in OVX rats treated with VEH produced no change (Zhang et al. 2008). In another study, the induction of DA by cocaine- and amphetamine-regulated transcript (CART), a protein the regulates mesolimbic function in response to stimulants (Kuhar et al. 2005), was enhanced by EST administration in OVX rats relative to OVX rats treated with VEH (Shieh and Yang 2008). Furthermore, this effect was attributed to an intracellular mechanism as only administration of EST that was permeable to cellular membranes facilitated CART-induced DA turnover (Shieh and Yang 2008). EST treatment also facilitated nicotine-evoked DA release in the striatum in female, but not male rats (Dluzen and Anderson 1997), suggesting that the effects of EST on stimulant-induced DA are sex specific.

4.2.3 Estrogen Receptor Subtype β

Facilitation by EST on the reinforcing effects of cocaine may be attributed to the interaction between ER-β and DA neurotransmission in the mesolimbic pathway. ER-β is found in DA neurons (Laflamme et al. 1998) and has been shown to influence DA receptor expression and neurotransmission (Morissette et al. 2008; Schultz et al. 2009) in the mesolimbic DA pathway. Furthermore, administration of the ER-β agonist DPN, but not the ER-α agonist PPT, reversed OVX-induced decreases in D_2 receptors and DA turnover within the striatum and NA core (Le Saux and Di Paolo 2006). ER-β also regulates cocaine-seeking behaviors. As previously noted, administration of the ER-β agonist DPN, but not the ER-α agonist PPT, enhanced cocaine-primed reinstatement (Larson and Carroll 2007) and amphetamine-induced CPP (Silverman and Koenig 2007) in OVX female rats. Also, administration of tamoxifen, a partial antagonist at ER-α, but pure antagonist at ER-β, reduced EST's-facilitating effects on the acquisition of cocaine

self-administration in EST-treated OVX female rats (Lynch et al. 2001). The contribution of ER-β to the rewarding effects of cocaine may be attributed to an intracellular mechanism involving downregulation of the regulator of G-protein signaling (RGS) 9-2. RGS9-2 regulates intracellular D_2 receptor activity and is highly localized in the NA following chronic cocaine exposure (Rahman et al. 2003; Wood 2007). Mice lacking this gene show enhanced responsiveness to cocaine (Rahman et al. 2003). Silverman and Koenig (2007) demonstrated that administration with an ER-β agonist, but not an ER-α agonist, reduced RGS9-2 expression in the core of the NA, a structure also implicated in cocaine-induced reinstatement (Ping et al. 2008), and enhanced amphetamine-induced CPP in OVX female rats. Together, these results suggest that EST enhancement of stimulant-related behaviors involves an interaction between ER-β and the mesolimbic DA pathway via intracellular transcription-related mechanisms that influence D2 receptor activity.

4.3 Progestins' Influence on the DA System

Far less work has been conducted on the effects of progestins on the mesolimbic DA pathway. However, the PROG metabolite, ALLO, attenuated stress-induced increases in DA (Dazzi et al. 2002), and altered DA release in the striatum and NA (Barrot et al. 1999; Jaworska-Feil et al. 1998; Laconi et al. 2007; Rouge-Pont et al. 2002). PROG also modulate DA levels in the striatum; however, results were equivocal and dependent on the time of testing, manner of administration, and presence or absence of EST (Dluzen and Ramirez 1984; 1987a,b; Fernandez-Ruiz et al. 1989). Thus, PROG and ALLO may interact with DA systems to influence drug-seeking behavior, but further work is needed to substantiate this.

4.4 Gamma-Aminobutyric Acid

An additional mechanism that may underlie EST and PROG's influence on cocaine seeking may be related to gamma-aminobutyric acid (GABA) neurotransmission. The facilitation and inhibition of GABA receptor neurotransmission resulted in the suppression and enhancement of mesolimbic DA, respectively (Tam and Roth 1985, 1990). Decreased GABA release was also associated with increased DA mediated behavior such as drug seeking in rats (Caille and Parsons 2004; Tang et al. 2005), while the administration of GABA receptor agonists decreased cocaine seeking (Campbell et al. 1999, 2002). This suggests that decreased GABA is associated with heightened vulnerability to cocaine-seeking behavior, whereas increased GABA may attenuate this behavior. Previous work indicated that EST inhibited activation of medium spiny GABAergic neurons in the striatum (Mermelstein et al. 1996), increased striatal GABA release (Hu et al.

2006), enhanced DA metabolism and turnover (Di Paolo et al. 1985; Shimizu and Bray 1993), and enhanced stimulant-elicited DA release in the striatum (Becker 1990a,b; Becker and Beer 1986; Castner et al. 1993). Conversely, PROG and its metabolites promoted striatal-GABA activity (Schumacher et al. 1989a,b) and decreased DA in the striatum (Dazzi et al. 2002; Dluzen and Ramirez 1987b; Jaworska-Feil et al. 1998; Laconi et al. 2007; Shimizu and Bray 1993). Thus, the different effects of EST and ALLO on cocaine seeking may be explained by their opposite effects on GABA and/or DA neurotransmission in areas of the brain that regulate drug seeking.

4.5 HPA

Stress is a major contributor to drug abuse (Fox and Sinha 2009), and activation of the stress system, the hypothalamic-pituitary-adrenal (HPA) axis, is associated with enhanced drug reward (Goeders 2002a,b). Preclinical work indicates that EST potentiated the release of CRF (Patchev et al. 1995; Swanson and Simmons 1989) and increased adrenocorticotropic hormone and corticosterone (Burgess and Handa 1992), which led to increased HPA activity (Dallman et al. 2004). These effects also extended to cocaine-induced HPA activation. In a study by Niyomchai and colleagues (2005), EST increased cocaine-induced corticosterone levels relative to VEH-treated controls. Behavioral studies provided further support for the role of EST on stress-related responses. EST facilitated fear-potentiated startle in OVX female rats relative to OVX rats treated with VEH, while PROG attenuated this facilitation (Hiroi and Neumaier 2006; Toufexis et al. 2004). Interestingly, ALLO attenuated HPA activation (Drugan et al. 1993; Frye et al. 2006; Owens et al. 1992; Patchev et al. 1994; Purdy et al. 1991), and it blocked stress-induced reinstatement in rats (Anker and Carroll, 2010). Further work is needed to examine the interaction between EST, the effects of stress, and behavior associated with drug abuse.

Overall, the neurobiological findings indicate that there are sex differences in DA receptor densities, intracellular DA neuronal activity, and extracellular DA levels in areas of the brain implicated in drug abuse vulnerability. Results presented in this chapter implicate EST in these differences possibly through its interaction with the ER-β. EST also decreases striatal GABA activation, and that may facilitate DA activation leading to increased sensitivity to the rewarding effects of drugs of abuse. Further, EST facilitates neurobiological and behavioral substrates of stress, which is a primary vulnerability factor in drug abuse. In contrast to EST, PROG and its metabolite ALLO exert an opposite effect on DA neurotransmission and potentiate GABA receptors and HPA activation that may lead to an attenuation of drug reinforcement.

In conclusion, the results indicate that females are more vulnerable to drug abuse than males during almost all of the critical phases of drug abuse: initiation, maintenance of rewarding effects, escalation of intake, extinction/craving, and

reinstatement/relapse. Females are also more responsive than males to a wide range of behavioral and pharmacological interventions that reduce drug taking and drug seeking. In contrast, males exhibited greater withdrawal effects than females, suggesting they are more sensitive to the aversive effects of drugs, while females are more responsive to the rewarding effects. These differences in responsiveness to the positive and negative effects of drugs are an emerging area of interest for medication development and other treatment approaches for drug abuse. The enhanced vulnerability to drug seeking in females may be attributed to an interaction between EST and the mesolimbic reward pathway, specifically the ER-β, and its interaction with DA neuronal activity. In contrast to EST, PROG and its metabolite ALLO exert an opposite effect on DA neurotransmission and potentiate GABA receptors that may attenuate drug reinforcement. Thus, EST and PROG differentially interact with the HPA axis, GABA, DA to influence sensitivity to the rewarding effects of drugs, and they are the basis of sex differences in drug abuse. The role of EST and PROG should be considered in the development of sex-specific prevention and treatment approaches for drug abuse.

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Sex Differences in Response to Stress and Expression of Depressive-Like Behaviours in the Rat

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Contents

Abstract Women are more susceptible than men to certain stress-related psychiatric disorders, such as depression. Preclinical studies aim to understand these sex differences by studying male and female rats in stress models. In this chapter, we review sex differences in behavioural aspects, as well as neurochemical and neurobiological findings derived from acute, repeated and chronic stress models. In particular, we focus on sex differences in depressive-like symptomatology expressed in the forced swim test, the chronic mild stress (CMS) and the learned helplessness models, the Flinders Sensitive Line rats (FSL), which is a genetic model of depression and in the lipopolysaccharide (LPS)-induced *sickness behaviour*, a putative inflammatory model of depression. Also, sex differences in stress effects

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on learning and memory parameters are discussed, because cognitive alterations are often seen in sex-differentiated psychiatric disorders. The observed behavioural alterations are often linked with abnormalities in the endophenotype, such as in hormonal, neurochemical, immune and neuroplasticity indices. From these data, it is clear that all stress models have strengths and limitations that need to be recognized in order to use them effectively in the investigation of sex differences in affective disorders.

Keywords Chronic mild stress · Depression · Flinders sensitive line · Forced swim test · Gender · Immunity · Learned helplessness · Learning · Serotonin · Sex differences · Sickness behaviour · Stress

Abbreviations

8-OHDPAT	(+)8-Hydroxy-N,N-dipropyl-2-aminotetralin hydrobromide
ACTH	Adrenocorticotropin hormone
AVP	Arginine vasopressin
CMS	Chronic mild stress
FSL	Flinders sensitive line
FST	Forced swim test
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamus-pituitary-gonadal
LPS	Lipopolysaccharide
LC	Locus coeruleus

1 Introduction

The organism's stress response is an evolutionary mechanism of great adaptive value; however, when stress exposure is prolonged and uncontrollable the response may be inadequate and maladaptive for the organism, presumably leading to physical and mental disorders, such as major depression, and anxiety disorders, which are major causes of disability in contemporary western societies (Lupien et al. 2009). Women are more susceptible than men to stress-related psychiatric disorders, such as major depression, generalized anxiety disorder, acute and post-traumatic stress disorder (Holden 2005). It is suggested that because of genetic, hormonal, biochemical and social factors, different responses to stressful life events and different coping strategies used by men *versus* women ultimately contribute to sex differences in the occurrence of stress-related disorders (Kendler et al. 2001; Maciejewski et al. 2001; Nemeroff et al. 2006; Sherrill et al. 1997). However, the basic neuroendocrine stress response, especially the one associated with acute stress, does not differ substantially between the two sexes (Klein and Corwin 2002).

Understanding sex differences in the stress response may contribute to the improvement of psychiatric diagnosis and treatment. Animal models have been widely used to study the effects of stress exposure on multiple aspects, such as hormonal, neurochemical, neurobiological and behavioural. Factors that determine the stress response include the type, the duration and the characteristics of the stressors, as well as the strain, the age of the animal and its genetic predisposition (Anisman and Matheson 2005). During the last decades, we have acknowledged the sex of the animal as an important factor that influences the stress response (Palanza 2001; Dalla et al. 2010). Although comparative studies on males and females are still limited, they have been conducted throughout the lifespan of the animal and include different protocols of stressor exposure, as well as genetic manipulations. Additionally, because of the role of stress as a predisposing and precipitating factor in the onset of depression (Anisman and Zacharko 1990; Holsboer 2001) and the marked sex differences in the prevalence of major depression in humans (Marcus et al. 2005), there has been a considerable interest in sex differences in depressive-like symptomatology expressed in several animal stress models. Other behavioural parameters that are studied and are often differentiated between the two sexes are cognitive, anxiety and activity responses. In particular, learning and memory parameters are widely studied in stress paradigms, because cognitive alterations are often seen in sex-differentiated psychiatric disorders, such as depression and post-traumatic stress disorder (Moore 2009; Sotiropoulos et al. 2008). In this chapter, we will review sex differences in the stress response, focusing on behavioural and neurochemical changes.

2 Sex Differences in Response to Acute and Repeated Stressful Experiences

2.1 Sex Differences in Neuroendocrine Responses

The organism's acute stress response mainly includes the activation of the locus coeruleus–norepinephrine (LC-NE) system and the hypothalamic-pituitary-adrenal (HPA) axis. The morphology of the LC is sexually dimorphic with females having a greater volume and number of neurons than males (Pinos et al. 2001). Also, its neuronal activity is more responsive to certain stressors in females than in males and this is probably regulated by corticotropin-releasing hormone (CRH) activity (Curtis et al. 2006).

On the other hand, HPA axis activation seems to be responsible for the slower response to stress. It is activated within minutes after the stressful stimulus and is initiated by the release of CRH and arginine vasopressin (AVP) by the hypothalamus (DeBold et al. 1984) and subsequently the release of adrenocorticotropin (ACTH) from the hypophysis. Such a cascade of events results in the release of glucocorticoids, such as corticosterone in rodents, from the adrenal cortex.

Under normal conditions, the secreted glucocorticoids inhibit further release of ACTH by feedback inhibition, thus enabling termination of the stress response and a return to homeostasis (Heinrichs and Koob 2004). This feedback is accomplished by the action of glucocorticoids on corticosteroid (GR and MR) receptors that are abundant in the limbic system, especially in the hypothalamus and hippocampus and their activation is sex-dependent (Karandrea et al. 2000, 2002). Sex differences are also evident in other aspects of HPA axis activity; female rats have higher resting levels of corticosterone and display greater diurnal changes in both ACTH and corticosterone than males (Handa et al. 1994; Kitay 1961). Also, in comparison to males, female rats have higher glucocorticoid levels following acute and repeated stress exposure and this seems to be dependent on circulating gonadal hormones (Galea et al. 1997; Seale et al. 2004a,b).

Prolonged activation of the HPA axis, as in the case of repeated or chronic stress exposure, can lead to "exhaustion" of the stress response system and halt the return to homeostasis. In this state, stress often has detrimental effects on the integrity and function of the brain. These effects include alterations in the activity of monoaminergic systems, in dendritic and synaptic remodelling in the hippocampus and frontal cortex, as well as in levels of adult hippocampal neurogenesis (Galea et al. 1997; McEwen 2002; Shors et al. 2007). In many cases, these changes have been linked to behavioural disturbances, such as depressive-like symptomatology and learning/memory deficits (Bekris et al. 2005; Bowman 2005; Dalla et al. 2005, 2008a; Kitraki et al. 2004; Shors 2006).

2.2 Sex Differences in Depressive Symptomatology

2.2.1 Swim Stress Exposure: Forced Swim Test

As mentioned, the HPA axis is activated in both sexes after acute or short-term stress exposure. For example, when male and female rats are exposed to two sessions of swim stress in two consecutive days (forced swim test; FST), corticosterone levels are enhanced in both sexes (Drossopoulou et al. 2004). At a behavioural level, both sexes increase their immobility levels during the second FST session, but females exhibit higher levels than males, which is indicative of increased despair and depressive-like symptomatology (Dalla et al. 2008a; Drossopoulou et al. 2004; Pitychoutis et al. 2009b) (Fig. 1). Accordingly, in the open space swimming test, which is a similar animal model to the FST, female rats appear more vulnerable to swim stress than males (Sun and Alkon 2006). During the FST, female rats also exhibit lower levels of head swinging behaviour than males and this has been linked to sex differences in postsynaptic serotonergic 5-HT_{2A} receptor activity (Darmani 1996; Drossopoulou et al. 2004; Matuszewich and Yamamoto 2003). At the neurochemical level, FST induces a decrease in serotonergic activity in the hippocampus and the hypothalamus of female rats, while serotonergic activity is increased in the hypothalamus of males. Moreover,

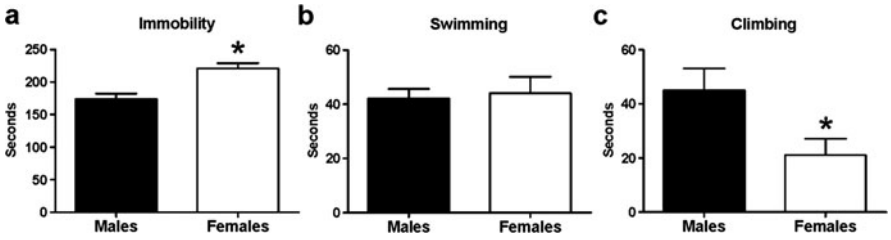

Fig. 1 *Behaviour of male and female rats during the Forced swim test*. Female rats spend more time immobile than male rats during the second session of the Forced swim test (FST), indicating enhanced levels of despair. Also, the duration of climbing, which represents an effort to escape from the cylinder, is higher in males than in females (* = $p < 0.05$) (Dalla et al. 2008a; Drossopoulou et al. 2004)

hypothalamic serotonin 5-HT_{1A} mRNA levels are decreased in female rats; while hippocampal 5-HT_{1A} mRNA levels are increased in males (Drossopoulou et al. 2004). Dopaminergic activity is also increased in the hippocampus and prefrontal cortex of male rats exposed to FST, while there is no effect in females (Dalla et al. 2008a). In both sexes, there is a tendency for enhanced GR mRNA levels 24h after the second swim session (Drossopoulou et al. 2004). However, following longer periods of repeated swim exposure, no alterations in GR or MR mRNA levels are detected in the female hippocampus and hypothalamus, while in males there is a down-regulation of GR mRNA in the hippocampus (Karandrea et al. 2002).

Thus, it seems that male rats have an adaptive response to swim stress by exhibiting enhanced corticosterone levels and increases in monoaminergic activity. On the other hand, female rats seem to be more vulnerable to swim stress than males and this is expressed by enhanced levels of despair and decreased serotonergic activity in the hippocampus and hypothalamus.

2.2.2 Shock Exposure: Learned Helplessness Model

In response to another kind of stressor (i.e. footshock or tailshock), males and females also exhibit elevated corticosterone levels (Heinsbroek et al. 1991). Numerous studies have shown that most males exposed to this kind of stress that they cannot control, develop helplessness behaviour, because when they are tested on a new task (e.g. avoid the footshock by going through a door-way at a shuttle box twice), they do not escape the stress even if they have the opportunity to do so (Maier 1984). This behaviour has been equated with a sense of "giving up", experienced by humans with major depression (Miller and Seligman 1975). In male rats, the expression of learned helplessness behaviour in operant-conditioning tasks, is accompanied by a wide range of physiological changes in response to shock, such as enhanced c-fos activity in the hippocampus, amygdala and prefrontal cortex, as well as monoaminergic activation in several brain regions and reduced rates of neurogenesis in the dentate gyrus of the hippocampus (Heinsbroek et al. 1990, 1991; Maier and Watkins 2005; Malberg and Duman 2003; Shors et al. 2007;

Trentani et al. 2003). When these stressors are repeated for longer periods and half of the rats can control the stressor by avoiding the shock exposure, corticosterone levels are still enhanced in all groups (i.e. in male rats exposed to controllable or uncontrollable stress) (Shors et al. 1989). However, only male rats, which are exposed to uncontrollable stress, exhibit learned helplessness behaviour, serotonergic activation and decreases in hippocampal neurogenesis (Amat et al. 2005; Bland et al. 2006; Heinsbroek et al. 1991; Shors et al. 2007).

Regarding females, although corticosterone levels and monoaminergic activity is also enhanced in female rats in response to footshock (Heinsbroek et al. 1991), most female rats do not express helplessness behaviour after acute or repeated uncontrollable footshock stress exposure (i.e. they learn to escape on the new task), while there is no effect of stress on neurogenesis levels in their hippocampus (Dalla et al. 2008b; Shors et al. 2007). Also, controllability over the stressor influences the degree of monoaminergic activation in a sex-dependent manner. In particular, the activation of noradrenaline and dopamine in the frontal cortex is larger after uncontrollable shock than after controllable shock and these differences are most evident in females. Moreover, uncontrollable shock induces higher serotonin levels in the frontal cortex of both sexes than controllable shock (Heinsbroek et al. 1991).

Notably, sex differences in learned helplessness behaviour cannot be attributed to gonadal hormonal differences, because they are not reversed by gonadectomy in adulthood of males or females (Dalla et al. 2008b) (Fig. 2). Sex differences in the learned helplessness model are probably mediated by sex differences in baseline escape behaviour in the operant-conditioning task, as well as in general activity levels. Indeed, unstressed female rats are in general more active and express less freezing behaviour than males (Dalla and Shors 2009; Padilla et al. 2009).

Overall, it seems that in this model, sex differences in the phenotype (i.e. behavioural sex differences in learned helplessness behaviour) do not always reflect sex differences in the endophenotype (e.g. corticosterone levels are equally affected in males and females by uncontrollable stress). However, levels of adult neurogenesis in the hippocampus seem to correlate with helplessness behaviour (Malberg and Duman 2003; Shors et al. 2007). Finally, controllability over the stressor seems to be an important factor, which determines the stress response in both sexes.

2.3 Sex Differences in Stress Effects on Learning

2.3.1 Associative Learning

Stress effects on different types of learning also differ between male and female rats. Regarding associative learning, exposure to an acute or repeated painful stressful event (i.e. tailshock, footshock or swim stress, but not restraint) greatly enhances learning during eyeblink classical conditioning in male rats, whereas the same stressor impairs learning in females (Shors 2006). Interestingly, animals that are exposed to the same amount of controllable shock do not express any change in this type of learning (Leuner et al. 2004). Corticosterone levels are

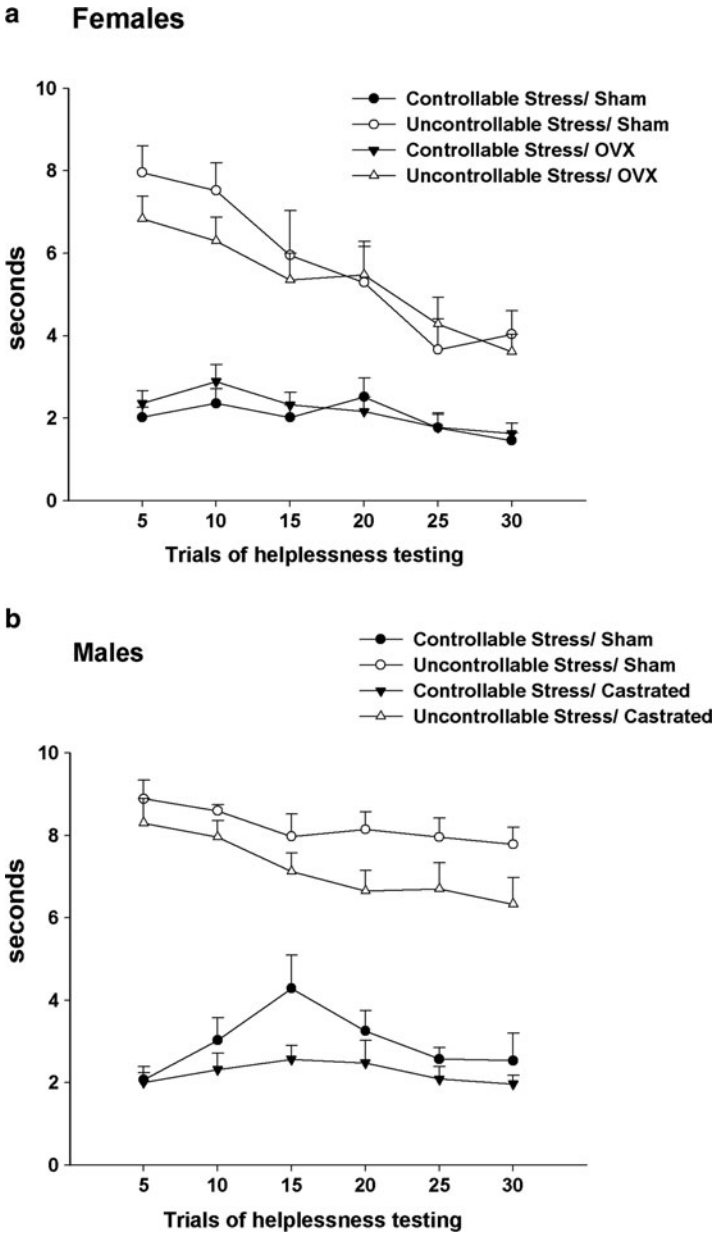

Fig. 2 *Males express learned helplessness behaviour whereas females do not*. During testing for learned helplessness behaviour, the rats have to cross a shuttle-box twice to escape the footshock. The graph depicts the escape latencies of male and female sham-operated and gonadectomized rats that were previously exposed to 7 sessions of controllable or uncontrollable footshock stress. Male sham- operated and castrated rats from the uncontrollable stress groups, did not learn to escape during testing and thus exhibited learned helplessness behaviour ($p > 0.05$). On the other hand, female sham-operated and ovariectomized (OVX) rats from the uncontrollable stress groups learned to escape during testing and did not exhibit learned helplessness behaviour ($p < 0.001$) (Dalla et al. 2008b)

enhanced in both sexes in response to the stressor, but adrenalectomy and gonadectomy studies have shown that males require the presence of glucocorticoids to enhance learning after stress, whereas females require the presence of ovarian hormones in order to impair learning after stress (Beylin and Shors 2003; Wood and Shors 1998). In particular, the impairment of associative learning after stress exposure emerges only in adult females with a mature oestrous cycle and is evident when females start training with eyeblink conditioning in proestrus when oestrogen levels are high (Shors et al. 1998).

Interestingly, the emergence of the stress effects on this type of associative learning in both males and females require an intact hippocampus (Bangasser and Shors 2007). Also, the same acute stressful experience increases the density of dendritic spines in the CA1 area of the male hippocampus and decreases it in the females, suggesting a link between density of spines in the hippocampus and associative learning (Shors et al. 2001, 2004). Notably, masculinized females injected with testosterone on the day of birth, exhibit enhanced learning during eyeblink conditioning and possess more spines in the CA1 area of the hippocampus after acute tailshock exposure (Bangasser and Shors 2008; Dalla et al. 2009).

Thus, females appear to be more vulnerable to shock exposure when associative learning and spine density in the hippocampus is evaluated. This is in contrast to the expression of helplessness behaviour that is mainly evident in male rats. The discrepancy between the two types of learning is probably due to inherent differences between the operant conditioning and the classical conditioning tasks. During operant conditioning that is used for assessment of learned helplessness behaviour, the animal must emit a voluntary motor response in order to change the outcome and learn, whereas during classical conditioning, which is indicative of associative learning, the animal emits an obligatory unconditioned response to the unconditioned stimulus, irrespective of volition (Shors 1998). Thus, it is not surprising that males and females respond differently in the two tasks and that sex effects are differently mediated by gonadal hormones.

2.3.2 Spatial Learning

In contrast to classical conditioning tasks, opposite effects of stress on performance of male and female rats in spatial learning and memory tasks have been repeatedly reported (Bowman 2005; Bowman et al. 2003). Following acute restraint stress, spatial memory in a Y-maze is impaired in males and is facilitated in females, irrespective of the oestrous cycle, while corticosterone levels are enhanced in both sexes (Conrad et al. 2004). Acute restraint stress has also been shown to enhance serotonergic activity in the basolateral amygdala of both sexes, but female rats show a greater response than males. Moreover, dopaminergic activity is increased in female rats, but not in males (Mitsushima et al. 2006). In the hippocampus, acute restraint stress significantly decreases brain-derived neurotrophic factor (BDNF) in both males and females in the CA3 area. In the dentate gyrus of the hippocampus, stress increases BDNF levels only in estradiol-treated ovariectomized rats, while it

decreases its levels in oestrogen-deprived rats (Franklin and Perrot-Sinal 2006). This finding can probably be linked with reduced levels of adult neurogenesis in the female hippocampus when oestrogen levels are low (Tanapat et al. 1999).

After one week of repeated restraint stress exposure, male rats are impaired in the object recognition test, while females exhibit enhanced performance during spatial memory tasks. These behavioural changes are accompanied by increased noradrenergic activity in the hippocampus and amygdala of females and decreased activity in males (Bowman et al. 2009). In the medial prefrontal cortex, the same protocol decreases the number and length of the apical neuronal dendritic branches of males, whereas in females, the same stress exposure increases apical dendritic length and this is prevented by ovariectomy (Garrett and Wellman 2009).

The detrimental effect of stress on the Morris water maze task is most evident in male rats exposed to longer periods of repeated restraint stress (21 days), while females exhibit improved memory scores (Bowman et al. 2003; Kitraki et al. 2004). Also, behavioural changes are accompanied by a decrease in GR receptor immunoreactivity in the male and an increase in the female hippocampus (Kitraki et al. 2004). The same stress protocol also decreases dopaminergic activity in the frontal cortex and amygdala of males but not females; whereas, in the hippocampus, stress increases levels of serotonin and noradrenaline in females, but not in males. These effects seem to depend on both organizational and activational effects of gonadal hormones (Luine 2002). Twenty one days of repeated restraint stress also induces apical dendritic atrophy of the CA3 pyramidal neurons in the male hippocampus, while this effect is not evident in females (Galea et al. 1997). However, spinophilin levels, which are indicative of new spine formation, are elevated in the male, but not female hippocampus following repeated restraint stress exposure (Khurana and Devaud 2007).

Thus, it seems that, in contrast to swim or shock stress exposure, males are more vulnerable to restraint stress than females, because they exhibit learning and memory deficits in spatial tasks, as well as decreases in monoamine levels and activity and decreases in certain neuroplasticity indices. On the other hand, females seem to adapt better to this type of stress exposure.

3 Sex Differences in the Effects of Chronic Mild Stress Exposure

Chronic mild stress (CMS) was developed in the late 1980s and is one of the most extensively investigated animal models of depression to-date. CMS's advantage over other models of depression lies on the fact that it employs relatively realistic inducing conditions (construct validity), simulates *anhedonia* which is a core symptom of major depression (face validity), and responds appropriately to antidepressant drugs (predictive validity) (Willner 1997, 2005; Willner et al. 1987). The CMS procedure involves continuous exposure to a variety of low-grade stressors, such as periods of food and water deprivation, small temperature

Table 1 Example of a weekly CMS protocol

Monday 10:00 h	Cage cleaning followed by no stress
Monday 20:00 h	Food and water deprivation for 14 h
Tuesday 10:00 h	Sucrose test, followed by food or water deprivation for 10 h
Tuesday 20:00 h	Paired housing for 14 h
Wednesday 10:00 h	Lights switched on and off every 2 h for 10 h
Wednesday 20:00 h	Soiled cage (250 ml of water was poured into the sawdust bedding) for 14 h
Thursday 10:00 h	Cage cleaning, followed by water deprivation for 10 h
Thursday 20:00 h	Paired housing for 14 h
Friday 10:00 h	Stroboscopic illumination in darkness for 10 h
Friday 20:00 h	Food deprivation for 14 h
Saturday 10:00 h	Tilting of the cages backwards (45 degrees) for 10 h
Saturday 20:00 h	Cages were put back in straight position/followed by no stress
Sunday 10:00 h	Stroboscopic illumination in darkness for 10 h
Sunday 20:00 h	Soiled cage (250 ml of water was poured into the sawdust bedding) for 14 h

reductions, changes of cage mates, and other similar mild manipulations (Table 1). Animals subjected to a battery of mild stressors for a period of several weeks develop a wide spectrum of behavioural, biochemical and physiological alterations, which can be effectively reversed upon chronic antidepressant treatment (Willner 1997, 2005). Exposure to a CMS regime ultimately leads to the induction of *anhedonia* (i.e. loss of pleasure derived from normally rewarding activities), which accounts for the impairment of rodents' preference for a palatable sucrose (or saccharin) solution.

Beyond *anhedonia*, this model simulates other symptoms of depression as well, such as decreased sexual behaviour and self-care, changes in sleep architecture and locomotor activity, as well as cardiovascular and immune alterations (Willner 1997, 2005). However, there have been some replication problems in CMS among laboratories (Willner 2005), mainly due to the failure of eliciting a significant decrease in sucrose consumption. This has been partly attributed to individual differences regarding rats' hedonic *status quo* (Duncko et al. 2003), as well as to differences between different strains of rats (Bekris et al. 2005; Konkle et al. 2003). Thus, it can be suggested that, besides sucrose consumption, we may be in need of more robust and extensive outputs to evaluate how CMS exerts its "*depressogenic*" influences on rats of both sexes. It is worth noting that the induction of *anhedonia* after CMS exposure has also been validated with the intracranial self stimulation paradigm, but inter-individual differences have also been reported (Nielsen et al. 2000). Moreover, the duration of stress application (e.g. 3 *versus* 7 weeks) and the stress regime itself (i.e. the cyclicity of the stressors) may also explain some of the discrepancies between different studies.

3.1 Sex Differences in Behavioural Responses

Sex differences in reward sensitivity are revealed when the CMS model of depression is applied to male and female rats. Rewarding reactivity to sucrose is impaired in both sexes (Duncko et al. 2001), but this phenomenon appears to be more robust

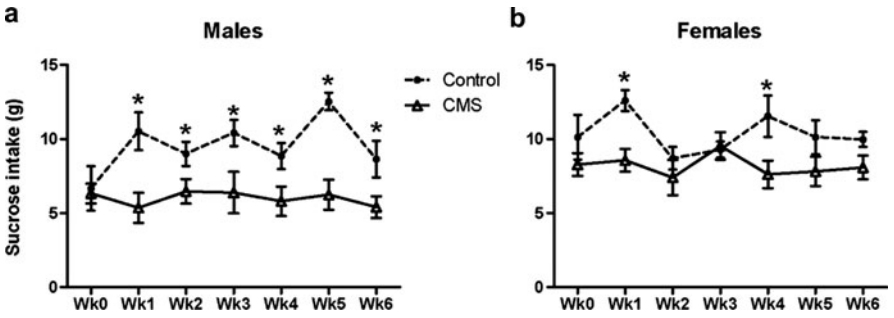

Fig. 3 *Sucrose intake in male and female rats during Chronic mild stress*. Male rats exposed to Chronic mild stress (CMS) consume a lower amount of a sucrose solution (1%) than control rats, during all weeks of CMS exposure. This effect is evident in female rats only during the first and fourth week of CMS exposure (* = $p < 0.05$) (Dalla et al. 2005)

in male compared to female CMS-exposed rats (Dalla et al. 2005, 2008a; Grippo et al. 2005; Kamper et al. 2009) (Fig. 3). As a matter of fact, it has been suggested that sucrose intake might not be an appropriate behavioural index for female rats, because unstressed females tend to drink more sucrose than males and show a more erratic increase in their consumption (Dalla et al. 2005). Interestingly, it has been shown that pair- or group- housing conditions may diminish the effects of CMS on body weight and sucrose consumption, in comparison to single-housed female rats (Baker and Bielajew 2007). On the other hand, another study that measured sucrose intake during 24 h periods has revealed a gradual reduction of sucrose consumption in female, but not in male CMS-treated rats (Konkle et al. 2003). However, no changes in thresholds for brain stimulation reward were observed in two strains of female rats (Baker et al. 2006).

As far as other behavioural measurements are concerned, female rats display less exploratory behaviour in a novel open field environment following three weeks of CMS application (Dalla et al. 2005). In another study, total activity in the open field test did not differ between stressed male and female rats, but CMS-exposed male rats exhibited enhanced locomotor activity during the first minute of the session, suggesting increased anxiety (Duncko et al. 2001).

3.2 Sex Differences in Neuroendocrine Responses

CMS application has been reported to dysregulate both the HPA and the hypothalamus-pituitary-gonadal (HPG) axes. For example, CMS induces severe disruptions of normal oestrous cyclicity. These alterations typically involve desynchronization of oestrous cycling in CMS-treated female rats that is either expressed by staying in one phase of the cycle or lengthening of the oestrous cycle (Baker et al. 2006; Dalla et al. 2005; Grippo et al. 2005; Konkle et al. 2003). However, in most cases when the stressful manipulations cease, the normal cyclicity is restored (Baker et al. 2006).

Impaired function of the HPA axis has repeatedly been noticed in patients with major depression (Holsboer 2000). As such, following, 3–4 weeks of CMS exposure both male and female rats displayed a tendency towards higher basal corticosterone levels (Duncko et al. 2001; Grippo et al. 2005). However, following 6 weeks of CMS exposure, a sex-specific elevation of corticosterone concentrations was observed only in female rats, suggesting that females are more vulnerable in the CMS model of depression than males (Dalla et al. 2005). Overall, it seems that there is an important time factor that differentiates corticosteroid responses between the two sexes upon chronic stress application.

3.3 Sex Differences in Neurochemical and Neurobiological Alterations

CMS exposure in male rats induces alterations in noradrenergic, dopaminergic and serotonergic *status*. In our initial studies in male rats, we observed an increase in dopaminergic and a decrease in serotonergic activity in the prefrontal cortex in two strains of male rats (*Sprague-Dawley* and *Wistar* rats) exposed to 7 weeks of CMS (Bekris et al. 2005). In the same study, we found a decrease in striatal dopaminergic activity and an increase in serotonergic activity in the hippocampus of male rats. All these effects were reversed by chronic imipramine treatment (Bekris et al. 2005). In further comparative studies between male and female rats, we used a milder CMS protocol, which did not induce neurochemical alterations in male rats, but resulted in a decrease in hippocampal serotonergic activity and prefrontocortical dopaminergic activity in females (Dalla et al. 2005, 2008a).

Accordingly, exposure to CMS induces a wide spectrum of relevant neurobiological alterations in specific brain regions implicated in the pathophysiology of major depression. For instance, it has been reported that hippocampal $5HT_{1A}$ receptors are increased by CMS in male rats (Papp et al. 1994). Four weeks of CMS exposure attenuates ACTH responses following systemic administration of a selective $5\text{-}HT_{1A}$ receptor agonist [(+)8-hydroxy-N,N-dipropyl-2-aminotetralin hydrobromide; 8-OH-DPAT] in both male and female rats (Grippo et al. 2005). These results were extrapolated as being indicative of CMS-driven alterations in $5\text{-}HT_{1A}$ receptor function in specific subpopulations of neurons in the central nervous system (Grippo et al. 2005). Also, it has been shown that CMS exposure is associated with a sex-specific enhancement of CRH mRNA levels in the hypothalamus of male rats (Duncko et al. 2001). Additionally, tyrosine hydroxylase mRNA levels in the LC were significantly decreased in response to CMS in both sexes, indicating an impairment of the central noradrenergic function (Duncko et al. 2001).

Thus, male and female rats are differentially affected by CMS application depending on the neurobiological indices that are measured. In some studies, females appear to be more vulnerable (e.g. serotonergic activity) while in other studies the two sexes are equally affected.

3.4 Sex Differences in Immune Parameters

CMS has long been associated with alterations in central and peripheral monoaminergic systems, as well as immunoreactivity (Willner 2005). CMS application has been shown to induce robust changes in thyroid hormone levels and reduction of the thymus weight in male rats (Kioukia-Fougia et al. 2002; Kioukia et al. 2000). In a recent study, we reported for the first time that both chronic clomipramine treatment and CMS application, exerted sexually dimorphic effects on cellular immunoreactivity [natural killer (NK) and lymphokine-activated killer (LAK) cell cytotoxicity and interleukin-2-induced T-cell proliferation], with female rats presenting a relatively immunosuppressed phenotype compared to males (Pitychoutis et al. 2009a). In addition, CMS and chronic clomipramine treatment induced sex-dependent alterations in the monoamine profile of the thymus. Thymic DA levels were augmented only in CMS-treated female rats with this increase being partially reversed by chronic clomipramine treatment. Further, while CMS application did not alter 5-HT and NA concentrations in either sex, chronic antidepressant treatment elevated thymic NA levels only in male rats, irrespective of stress application (Pitychoutis et al. 2009a). Intriguingly, clomipramine treatment rendered thymic 5-HT a positive modulator of LAK cytotoxicity; thymic monoamine alterations being associated with functional measures of cellular immunity are suggestive of a thymus-dependent route by which antidepressants could affect cell-mediated immunity (Pitychoutis et al. 2009a; Pitychoutis et al. 2010).

4 Sex Differences in Sickness Behaviour, a Putative Inflammatory Model of Depression

Data accumulated largely during the last two decades advocate the innate immune response as a mechanism that may be implicated in the pathophysiology of major depression, mainly due to the documented alterations in the ability of immune cells to secrete proinflammatory cytokines (Dantzer et al. 2008). Challenging the innate immune machinery with the proinflammatory agent lipopolysaccharide (LPS) induces a mild state of *nosothymia*, termed as *sickness behaviour*. *Sickness behaviour* is conceptualized through numerous depressive-like behavioural and physiological manifestations, most of which overlap with the clinical symptoms of depression (i.e. *anhedonia*, depression in motor/exploratory activity and a reduction in food intake, activation of the HPA axis, as well as alterations in central and peripheral monoamine utilization) (Dantzer et al. 2008; Zampeli et al. 2009).

Behavioural responses in LPS/cytokine-induced sickness have been studied in both male and female rats in only a few studies. Female rats exhibit greater sensitivity than males to LPS and/or cytokine administration in several aspects of behaviour, including sexual activity and sucrose reward (Avitsur and Yirmiya 1999; Merali et al. 2003). However, *in vitro* experiments have shown that

LPS-challenged macrophages derived from male mice produced higher levels of inflammatory cytokines than similarly treated female-derived cells, suggesting that males may be more susceptible to bacterial sepsis than females (Marriott et al. 2006). Furthermore, female rats develop behavioural tolerance to repeated LPS administration more quickly than males and this phenomenon was found to be oestrous cycle-dependent (Engeland et al. 2006).

Traditional measures of sickness (i.e. impairment of social exploration of a juvenile conspecific and weight loss) have been shown to be equally affected in male and female rats, except for *anorexia* (lack of appetite for food) establishment where males appeared to be more vulnerable (Pitychoutis et al. 2009b). Sickness establishment also alters the way the two sexes react upon stress exposure. In particular, LPS administration (100 μg/kg) induced a beneficial female-specific enhancement of coping ability in the stressful FST, as evidenced by the increased swimming durations achieved during the second swim session induced (Pitychoutis et al. 2009b). However, it has been shown that higher doses of LPS (e.g. 2 mg/kg) reliably induced an increase in floating time in rats of both sexes, at 24 h post-injection (Tonelli et al. 2008). Finally, *anhedonia*, which is a core symptom of depression, was equally established in both sexes at 24 h post-LPS administration, with this effect being reversed within the following day (Pitychoutis et al. 2009b) (Fig. 4).

Ex vivo neurochemical analysis at 2 h post-LPS administration, when many symptoms of sickness reach a *plateau*, indicated that central serotonergic activity in female rats is enhanced in all limbic sites examined (i.e. hypothalamus, hippocampus, prefrontal cortex, hippocampus, amygdala and striatum). On the other hand, serotonergic *status* in male rats was only modestly altered (Pitychoutis et al. 2009b). Dopaminergic indices were primarily affected in female rats, especially in the striatum, while there were no apparent alterations in males following LPS administration. In addition, neuroendocrine corticosteroid responses further confirm that females are more vulnerable to LPS sensitization compared to males

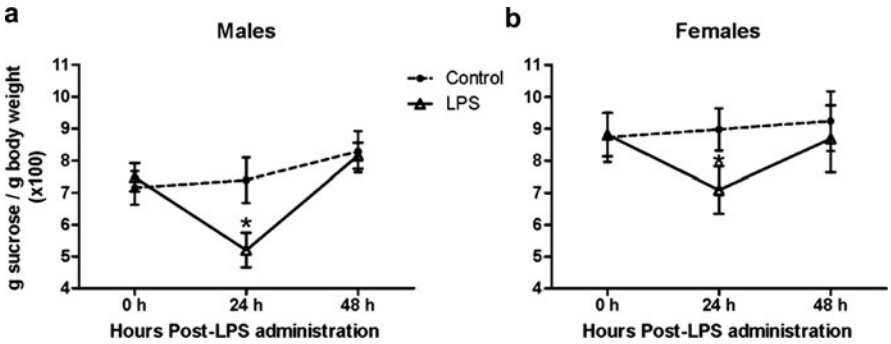

Fig. 4 *Sucrose intake after Lipopolysaccharide (LPS) administration*. Anhedonia was established equally in rats of both sexes at 24 h post-LPS (100 μg/kg, ip) administration, as evidenced by the relative consumption of a sucrose solution (1%). This effect was reversed within the following day (* = $p < 0.05$) (Pitychoutis et al. 2009b)

(Frederic et al. 1993; Pitychoutis et al. 2009b; Spinedi et al. 1994; Tonelli et al. 2008).

The apparent sex differences underlying the neurochemical and behavioural profile of *sickness behaviour* point to the important role of the immune system activation in the pathophysiology of depression. Taking into account the higher prevalence of affective disorders in females, a focus on the basic science of sex differences that underlie *sickness behaviour* is useful in delineating the neuroimmunological substrate for the appearance, course and outcome of these conditions (Pitychoutis and Papadopoulou-Daifoti 2010).

5 Sex Differences in a Genetic Model of Depression: Flinders Sensitive Line

Current evidence points towards a gene-environment interaction in the ability to cope with stress and with the predisposition to stress-related disorders, such as depression (Cryan and Slattery 2007). In agreement with this, many laboratories study genetic models of depression, such as the *Flinders Sensitive Line* (FSL) of rats. These rats have been created by selectively breeding *Sprague-Dawley* rats for their hyper-sensitivity to cholinergic agonists; a characteristic that has also been observed in depressed humans (Overstreet et al. 2005). FSL rats present a number of behavioural similarities to depressed individuals, such as reduced general activity, disturbed appetite, REM-sleep abnormalities and *anhedonia* (Yadid et al. 2000). Also, FSL rats display a clear depressive-like behavioural profile, particularly showing increased immobility, already observed from the first swim session of the FST paradigm. Chronic treatment with different antidepressant agents successfully reverses the depressive-like behaviour of male FSL rats (Kokras et al. 2009a; Overstreet et al. 2005). Regarding females, we have found that they do not exhibit enhanced immobility levels, but they show decreased latency to become immobile, in comparison to *Sprague-Dawley* controls. Interestingly, sex differences in FST performance are alleviated following treatment with the tri-cyclic antidepressant, clomipramine (Kokras et al. 2009a) (Fig. 5). Sex differences are also present in the frequency of head swinging behaviour, with female FSL rats exhibiting lower levels than males, as do *Wistar* rats (Drossopoulou et al. 2004). However, antidepressant treatment does not affect head swinging behaviour and sex differences are still apparent after treatment (Kokras et al. 2009a).

Male FSL rats exhibit several neurochemical abnormalities, including serotonergic alterations, increased levels of catecholamines and their metabolites, and impaired communication between serotonergic and dopaminergic systems in limbic brain regions (Kokras et al. 2009a; Yadid et al. 2000). In male FSL rats at baseline, serotonergic activity, as indicated by serotonin turnover, is lower in limbic regions, compared to *Sprague-Dawley* controls, while it is modestly increased after chronic

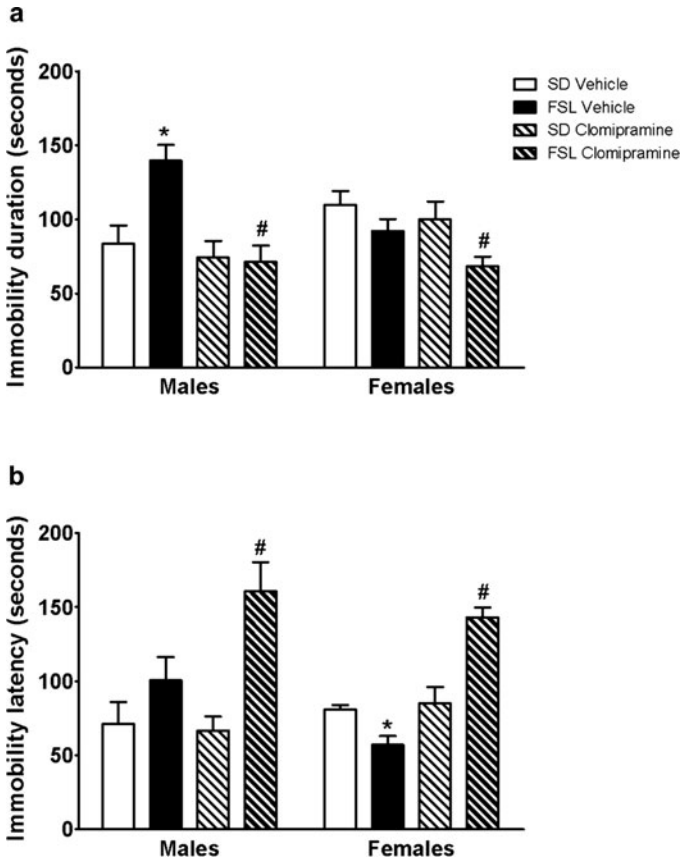

Fig. 5 *Behaviour of male and female Flinder rats during Forced swim test*. Male *Flinders Sensitive Line* rats (FSL) exhibited increased immobility duration during one session of FST, in comparison to *Sprague-Dawely* (SD) rats, which serve as controls. Female FSL rats exhibited reduced latency to become immobile during one session of FST, in comparison to SD rats, which serve as controls. Chronic clomipramine treatment reversed the immobility duration and latency in both sexes (*,# = $p < 0.05$) (Kokras et al. 2009a)

treatment with a tri-cyclic antidepressant (Kokras et al. 2009a). Interestingly, this is not the case for female FSL rats, which do not differ in serotonergic activity from their control *Sprague-Dawley* rats. However, clomipramine treatment results in a marked increase in serotonergic activity of female FSL rats in all brain regions tested (Kokras et al. 2009a). Also, clomipramine treatment increases cortical glutamate levels in both sexes and hippocampal glutamate only in female FSL rats (Kokras et al. 2009b). These findings indicate that antidepressant treatment may alleviate sex differences in the phenotype (i.e. converging effects on behaviour during FST) while maintaining or intensifying sex differences in the endophenotype (i.e. serotonergic activity).

6 General Conclusions

Overall, it seems that the type and the duration of stressor, as well as the behavioural parameters that we measure, influence the appearance and direction of sex differences in stress response. Depressive-like symptomatology is also expressed differently between the two sexes depending on the model. For example, although female rats appear to be more vulnerable in the FST and CMS models and following LPS administration, they do not exhibit learned helplessness behaviour in response to footshock.

In most cases, behavioural alterations can be linked with abnormalities in the endophenotype, such as in hormonal, neurochemical, immune and neuroplasticity indices. In particular, the HPA axis is activated in both sexes after stress exposure, but some components are differentiated between the two sexes pointing to its importance in the emergence of stress-related disorders, such as depression. Also, monoamines are generally activated after stress exposure, but in some cases, especially in females, we observe decreases in monoaminergic activity in response to stress, which may be linked with the higher incidence of depression in women (Deecher et al. 2008). Moreover, sex differences are mediated in a complex way by the effects of gonadal hormones during development of the brain (organizational effects), during puberty and in adulthood (activational effects).

Finally, it can be hypothesized that certain stress models can lead to decreases in serotonergic activity in the hippocampus of female rats, which in turn can be linked with decreased neuroplasticity associated with cognitive impairment and depressive-like symptomatology. However, more studies focusing on animal models validated for females are needed in order to evaluate this hypothesis.

Overall, it is clear that all the stress models have strengths and limitations that need to be recognized in order to use them effectively in the investigation of affective disorders. Limiting research on depression to male animals, may lead to inaccurate findings or hide important results that apply only to women. Also it is possible that sex hormone's actions on the brain from "womb to tomb" may affect the brain in a sexually dimorphic manner. This could lead to increased vulnerability to stressors, different coping strategies and differentiated responsivity to antidepressants in women.

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Importance of the COMT Gene for Sex Differences in Brain Function and Predisposition to Psychiatric Disorders

Elizabeth M. Tunbridge and Paul J. Harrison

Abstract As outlined elsewhere in this volume, sex differences can affect brain function and its dysfunction in psychiatric disorders. It is known that genetic factors contribute to these sex dimorphisms, but the individual genes have rarely been identified. The catechol-*O*-methyltransferase (COMT) gene, which encodes an enzyme that metabolises catechol compounds, including dopamine, is a leading candidate in this regard. COMT's enzyme activity, and the neurochemistry and behaviour of COMT knockout mice are both markedly sexually dimorphic. Furthermore, genetic associations between COMT and psychiatric phenotypes frequently show differences between men and women. Although many of these differences are unconfirmed or minor, some appear to be of reasonable robustness and magnitude and are reviewed in this chapter. Sexually dimorphic effects of COMT are usually attributed to transcriptional regulation by oestrogens; however, a careful examination of the literature suggests that additional mechanisms are likely to be at least as important. Here, we review the evidence for a sexually dimorphic influence of COMT upon psychiatric phenotypes and brain function, and discuss potential mechanisms by which this may occur. We conclude that despite the evidence being incomplete, there are accumulating and in places compelling data showing that COMT has markedly sexually dimorphic effects on brain function and its dysfunction in psychiatric disorders. Although oestrogenic regulation of COMT is probably partially responsible for these sex differences, other mechanisms are likely also involved. Since sex differences in the genetic architecture of brain function and psychiatric disorders are the rule not the exception, we anticipate that additional evidence will emerge for sexual dimorphisms, not only in COMT but also in many other autosomal genes.

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As the contributions to this volume make clear, sex is a key factor in neuroscience (Cahill 2006) and psychiatry (Cosgrove et al. 2007). Many aspects of brain function and structure vary between men and women (Gur et al. 1995; Murphy et al. 1996; De Courten-Myers 1999; De Bellis et al. 2001; Goldstein et al. 2001; Preece and Cairns 2003; De Vries 2004), and most psychiatric disorders show sex differences in one or more variables including incidence, age at onset, clinical features, and outcome (Lensi et al. 1996; Tamminga 1997; Piccinelli and Wilkinson 2000; Aleman et al. 2003; Baron-Cohen et al. 2005). These dimorphisms are usually assumed to result from the effects of sex hormones (Collaer and Hines 1995; Rubinow and Schmidt 1996; Seeman 1997; Kelly et al. 1999), as well as to the actions of genes located on the sex chromosomes (Vawter et al. 2004; Cutter et al. 2006; Davies and Wilkinson 2006). However, sex differences in epigenetic mechanisms, such as DNA methylation and chromatin modifications, relevant to brain function may also play a major role (Kaminsky et al. 2006). Furthermore, there is also evidence that autosomal genes contribute to sex differences in the genetic predisposition to psychiatric phenotypes. For example, sex differences have been found in the chromosomal loci implicated in the vulnerability to major depression (Holmans et al. 2004; Nash et al. 2004), neuroticism (Fullerton et al. 2003), obsessive–compulsive disorder ([OCD]; Nestadt et al. 2000), and autism (Stone et al. 2004). These differences occur in addition to sex differences in the heritability estimates for certain disorders; for example, major depression is estimated to be more heritable in women than men (Kendler et al. 2006). Taken together, these indications that there are differences in the genetic factors contributing to psychiatric phenotypes in men and women are consistent with the fact – not always appreciated – that the genetic basis of many, perhaps most, human traits is sexually dimorphic (Weiss et al. 2006).

Although, as described above, a diverse set of evidence demonstrates that there are sex differences in the genetic modulation of normal brain function and psychiatric disorders, few candidate genes for mediating these sex differences have been described. Here, we outline evidence that COMT is one such gene and consider the mechanisms by which its sexually dimorphic effects on brain function may be mediated. This chapter is an updated version of, and revised from, a recent review (Harrison and Tunbridge 2008).

1 Catechol-*O*-methyltransferase

The neurobiology and pharmacology of COMT have been reviewed in detail elsewhere (Weinberger et al. 2001; Tunbridge et al. 2006a; Mannisto and Kaakkola 1999); however, a brief introduction is given below. The COMT enzyme metabolises catechol-containing compounds, including dopamine and noradrenaline and,

notably with respect to sex differences, catechol oestrogens. The COMT gene exists in at least two protein isoforms produced from alternate start codons: membrane-bound COMT (MB-COMT) and soluble COMT (S-COMT), which differ in their capacity and affinity for different catechols. In contrast to the dominance of S-COMT in most peripheral tissues, MB-COMT is the most abundant isoform in brain, consistent with its high affinity for the catecholamine neurotransmitters. The relevance for COMT in regulating brain function and risk for psychiatric disorders arises primarily from its key role in regulating cortical dopamine (Tunbridge et al. 2004a; Yavich et al. 2007). However, as outlined below, COMT's role in metabolising catechol oestrogens, a function which is generally overlooked from a neurobiological perspective, may be important for mediating some of its sexually dimorphic effects.

The COMT gene is located on chromosome 22q11. It contains several single nucleotide polymorphisms (SNPs) of known or suspected functional significance. The best studied of these is rs4680, a 472 G/A substitution at codon 158 in MB-COMT (codon 108 in S-COMT) that encodes either the ancestral valine (Val^{158}) or methionine (Met^{158}), which we refer to as $Val^{158}Met$. The $Val^{158}Met$ SNP directly affects the thermal stability of COMT; therefore, at 37°C the Val^{158} form is more stable, and thus more active, than the Met^{158} form. In human prefrontal cortex, this substitution results in an approximately 40% reduction in Met/Met homozygotes, compared with Val/Val homozygotes, with heterozygotes showing intermediate COMT enzyme activity (Chen et al. 2004). Earlier studies reported even greater differences in erythrocyte COMT activity between Val/Val and Met/Met carriers (Lachman et al. 1996), although this might, in part, result from methodological differences between the studies. Nevertheless, the $Val^{158}Met$-encoded enzyme activity differences, coupled with the importance of COMT for regulating cortical dopamine levels, suggest that cortical dopamine signalling is likely to be enhanced in Met^{158}- compared to Val^{158}-carrying individuals. Following the landmark study of Egan et al. (2001), a range of studies have shown that the $Val^{158}Met$ allele has a small but significant impact on working memory, attention and executive performance, and prefrontal efficiency, with Met^{158}-carrying individuals performing better and/or more efficiently than Val^{158}-carrying individuals (Barnett et al. 2007b; Mier et al. 2009). Conversely, the Val^{158} allele is associated with more "flexible" cognitive responses (Bilder et al. 2004), something which may be more adaptive during emotional processing (Smolka et al. 2005; Drabant et al. 2006). Apart from $Val^{158}Met$, most other common COMT SNPs are non-coding (synonymous, intronic, or in the 5′ or 3′-untranslated regions). As such, any functional impact they confer is presumably mediated by an effect on COMT expression. However, direct evidence for this hypothesis remains unclear (Bray et al. 2003; Chen et al. 2004; Tunbridge et al. 2004b; Dempster et al. 2006) and is likely complicated by non-linear interactions between SNPs within COMT (Meyer-Lindenberg et al. 2006; Nackley et al. 2006). An elegant demonstration of this was provided by Nackley et al. (2006), who demonstrated that different COMT haplotypes can result in different COMT mRNA secondary structure, thereby affecting its mRNA stability, protein expression, and enzyme activity in a manner

that could not be predicted by examining any one of the haplotype-comprising SNPs individually. In any event, the majority of studies informing of sexually dimorphic effects of COMT have studied Val^{158}Met; therefore, this polymorphism is the main focus of this review.

2 Sexual Dimorphisms in COMT Function

COMT exhibits sexual dimorphisms in the normal brain that set the scene for, and may contribute to, differences in its involvement in psychiatric disorders between men and women. An important study by Chen et al. (2004), involving post-mortem brain tissue from 118 subjects, showed COMT activity in the prefrontal cortex to be 17% higher in men than women (independent of Val^{158}Met and other SNPs). These findings are in agreement with earlier studies that demonstrated approximately 30% higher enzyme activity in men in liver (Boudikova et al. 1990) and also in most (Fahndrich et al. 1980; Floderus and Wetterberg 1981; Philippu et al. 1981) but not all (Fitzgerald et al. 1980) studies of erythrocytes. It is not known at what stage in life the sex difference in brain COMT activity manifests, since the sole developmental study was conducted almost entirely in males (Tunbridge et al. 2007a). Interestingly, the higher brain COMT activity in men occurs despite levels of COMT protein and mRNA being similar in both sexes (Bray et al. 2003; Chen et al. 2004; Tunbridge et al. 2004b) or perhaps even higher in women (Dempster et al. 2006). This dissociation between expression and activity has implications for the likely mechanism underlying COMT's sexual dimorphisms, as we discuss later.

The other primary evidence for sexual dimorphism in normal COMT function comes from examination of neurochemical function and behaviour of the COMT knockout mouse (Gogos et al. 1998). Tissue dopamine levels in the frontal cortex are increased almost threefold in male COMT −/− mice (and twofold in +/− mice) compared to wild-type mice, confirming the importance of COMT in cortical dopamine metabolism; conversely, in female COMT +/− and −/− mice, dopamine levels were unchanged. The lack of effect of COMT deletion on frontal cortex dopamine in female mice presumably reflects the existence of sex-specific compensatory mechanisms (such as a higher activity of dopamine or noradrenaline transporters), although this has not been determined. Male, but not female, COMT knockout mice perform better on certain memory tests, showing higher levels of spontaneous alteration and better performance on the Barnes maze task than wild-type mice (Babovic et al. 2007). Similarly, male but not female knockout mice also have a significantly higher preference for ethanol, compared with their wild-type littermates (Tammimäki et al. 2008). In contrast, female but not male COMT null mice showed greater anxiety compared to wild-type mice (Gogos et al. 1998). These latter data suggest that COMT deletion is still of functional significance in female mice, despite the lack of an alteration in frontal dopamine. It is plausible that the anxiety changes in female COMT knockout mice results from the action of COMT in the hippocampus, a brain region which is critical for modulating anxiety

phenotypes (Gray and McNaughton 2000) and in which COMT is highly expressed. However, the role of COMT in the hippocampus remains to be investigated. Further studies in the COMT knockout mice, using cocaine, GBR 12909 (a dopamine transporter inhibitor), levodopa, or amphetamine to pharmacologically modulate dopamine levels, showed that some but not all neurochemical and behavioural responses to these pharmacological challenges are sex-specific (Huotari et al. 2002a,b, 2004; see also O'Tuathaigh et al. 2007). For example, D-amphetamine administration affected dopamine metabolism equally in male and female knockout mice (Huotari et al. 2004), whereas hyperactivity in response to GBR 12909 was attenuated only in males (Huotari et al. 2002b).

A final noteworthy COMT sexual dimorphism concerns the potential for sex differences in allele frequencies. In 4,014 Ashkenazi Jews (control subjects participating in a genetic association study of schizophrenia discussed below), Shifman et al. (2002) found that the frequency of the A allele of the 3′-untranslated region SNP rs165599 was higher in women than men (65% vs. 61%, $p = 0.0009$); they state that a similar difference was seen in a second sample, giving a combined p value of 0.00009 (see also Shifman et al. 2004). The authors speculate that the finding may be due to a reduced viability of male foetuses carrying this allele. However, to our knowledge, this sex difference in rs165599 allele frequency has not been replicated (Sweet et al. 2005; see also Molero et al. 2007) – albeit no other study has reported on such a large sample – and so its significance remains unclear.

3 Sexually Dimorphic Effects of COMT Genotype on Brain Function and Psychiatric Disorders

Listed in Table 1 are many (but not all) of the studies that have reported a sex difference in the impact of COMT genotype on a cognitive or psychiatric phenotype. Patsopoulos et al. (2007) highlight that the majority of articles claiming sex differences fail to provide satisfactory evidence to support this claim. In part consistent with this assertion, the studies listed vary in the robustness and plausibility of the finding with regard to sex. Few have been statistically convincing, for example, by demonstrating a sex-by-genotype interaction in analysis of variance (ANOVA; e.g. Kates et al. 2006; Lang et al. 2007). Many studies simply found an association that reached significance in one sex but not in the other (e.g. Enoch et al. 2006). Findings of a significant difference in one sex and not in the other were often further compromised by the fact that the non-significant sex had a smaller sample size and therefore had less power to detect an effect, compared with the significant sex (e.g. Nolan et al. 2000; Ono et al. 2004; Stein et al. 2005). Other studies discuss sex-related effects despite reporting a non-significant sex-by-genotype ANOVA interaction (e.g. Olsson et al. 2005; Zinkstok et al. 2006), while a final category of studies, not included in the table, provide only trend-level findings of sex-related effects of COMT genotype (e.g. Eley et al. 2003; Sazci et al. 2004; Woo et al. 2004).

Table 1 Chronological list of studies reporting sexually dimorphic effects of COMT genotype on psychiatric disorders and allied phenotypes[a]

Citation	Phenotype/parameter	Male/females	Finding related to sex
Karayiorgou et al. (1997)[b]	OCD	117/104	Met^{158} allele associated in men ($p = 0.0002$) not women ($p = 0.066$)
Nolan et al. (2000)	Suicide attempts in schizophrenia	117/31	Met^{158} allele associated in men ($p = 0.028$) not women ($p > 0.5$)
Dauvilliers et al. (2001)	Sleep latency in narcolepsy	59/38	Longer latency associated with Met^{158} allele in men, Val^{158} allele in women
Shifman et al. (2002)[b]	Schizophrenia	3,980/1,643	G allele of rs165599 associated in women ($p < 0.00001$) not men ($p = 0.1$). Sex difference $p = 0.01$
Enoch et al. (2003)	Harm avoidance	160/241	Met^{158} homozygosity associated in women ($p \leq 0.03$) not men ($p \geq 0.79$)
Qian et al. (2003)	ADHD	170 case, 376 control/18 case, 17 control	Met^{158} associated with ADHD in males ($p = 0.05$; family-based study), while Val^{158} associated in females ($p = 0.044$; case control)
Domschke et al. (2004)	Panic disorder	82/148	Val^{158} allele associated in women ($p = 0.01$) not men ($p = 1.0$)
Ono et al. (2004)	Suicide	112/51	Val^{158} homozygosity protective in men ($p = 0.016$) not women ($p = 0.96$)
Olsson et al. (2005)	Persistent episodic anxiety	340/473	Met^{158} homozygosity associated in women ($p = 0.02$) not men ($p = 0.38$). No genotype × sex interaction
Poyurovsky et al. (2005)	OCD	109/141	Met^{158} allele associated in men ($p = 0.029$) not women ($p = 0.78$)
Stein et al. (2005)	Low extraversion	154/343[c]	Met^{158} homozygosity associated in women ($p = 0.001$) not men ($p = 0.6$)
Sweet et al. (2005)	Psychosis in Alzheimer's disease	130/243[c]	Val^{158} allele associated in women ($p = 0.005$) not men ($p = 0.383$)
Kates et al. (2006)[b]	Dorsal and orbital frontal volumes	25/26	Opposite effects in boys and girls with VCFS[d]. Sex x genotype interaction ($p < 0.001$)
Beuten et al. (2006)[b]	Nicotine dependence	668[e]/1369[c,e]	Met^{158} allele associated in women. Sex × genotype interactions ($0.006 < p < 0.02$)
Denys et al. (2006)	OCD	135/170	Met^{158} allele associated in men ($p = 0.036$) not women ($p = 0.23$)
Enoch et al. (2006)	Alcoholism and smoking	141/201	Val^{158} allele associated in women ($p = 0.011$) not men ($p = 0.186$)
Kim et al. (2006)	Harm avoidance	138/148	Val^{158} allele associated in women ($p = 0.003$) not men ($p = 0.36$)
O'Hara et al. (2006)	Delayed verbal recall tests	62[f]/101[f]	Val^{158} allele associated with 8 point WMS-R[g] advantage in men. Sex × genotype interaction on BNT[h]
Rothe et al. (2006)	Panic disorder	60/118[e]	Val^{158} allele associated in women ($p = 0.008$) not men ($p = 0.272$)
Rybakowski et al. (2006)	WCST[i] in schizophrenia	43/36	Val^{158} homozygosity associated with fewer errors by men ($p = 0.044$), more errors by women ($p = 0.042$)
Zinkstok et al. (2006)	Grey and white matter volumes	57/97	Ageing effects related to genotype in women only. No genotype × sex interactions
Barnett et al. (2007a)[b]	IQ and executive function	2,650[j,k]/2,650[j,k]	Effects in boys only. Genotype × sex interactions on attention and verbal IQ

Baud et al. (2007)	Trait anger in suicide attempters	211/536	Val^{158} homozygosity associated with higher trait anger in women ($p = 0.002$) but not men
Domschke et al. (2007)[b]	Panic disorder	209/319	Meta-analysis. Association in women only: Val allele in Caucasians, Met allele in Asians
Golimbet et al. (2007)	Novelty seeking	56/74	Met^{158} homozygosity associated with higher trait scores in women ($p = 0.018$) not men
Lang et al. (2007)[b]	Sensation seeking	214/218	Val^{158} homozygosity associated in women ($p \geq 0.005$) not men[l]. Sex × genotype interaction ($p = 0.005$)
Ma et al. (2007)	Schizotypy in healthy volunteers	231/234	Val^{158} homozygosity associated with lower schizotypy scores in men ($p = 0.009$) but not women
Pooley et al. (2007)[b]	OCD	580/718	Meta-analysis. Met^{158} allele associated in men ($p = .001$) not women ($p = 0.83$). Sex difference $p = 0.0001$
Weiss et al. (2007)	Emotional recognition	49/51	Met^{158} homozygosity associated with impaired recognition of sad faces in women ($p = 0.03$) not men
Zhang et al. (2007)	WISC[m]	142/163	Freedom from distractibility scores associated in girls (p-0.03; 0.06 after Bonferroni) but not boys
Barnett et al. (2008)	Meta-analysis of Val^{158}Met and cognitive measures		N-back: negative association between study effect size and proportion of males ($p < 0.001$). Verbal recall: positive association between study effect size and number of male participants ($p = 0.007$)
Biederman et al. (2008)	ADHD[n]	308/166	Met^{158} allele associated in males ($p = 0.003$) but not females. Sex effect: $p = 0.071$; significance increases to $p = 0.007$ when combined with Qian et al. (2003)
Oosterhuis et al. (2008)	Opiate dependence	11 case, 50 control/18 case, 43 control	Met^{158} associated with dependence in female Hispanics ($p = 0.049$). Does not survive multiple testing correction and not found in other ethnicities
Pelayo-Terán et al. (2008)	Schizophrenia clinical characteristics	63/40	Val^{158} associated with longer duration of untreated psychosis in females ($p = 0.011$) but not males. Sex × genotype interaction: $p = 0.011$
Talkowski et al. (2008)[b]	Schizophrenia	Not stated (478 cases, 501 controls)	rs737865 associated in women ($p = 0.008$) but not men. Sex × genotype interaction: $p = 0.0007$
Barnett et al. (2009)[b]	IQ and cognition	4211/3962	COMT haplotype associated with verbal IQ in boys but not girls. Sex × genotype interaction ($p = 0.03$). Working memory span associated with rs165599 in boys but not girls. Sex × genotype interaction: $p = 0.02$
Domschke et al. (2009)	Therapeutic response to ECT[o]	33/71	Val^{158} allele associated with better response in females ($p = 0.016$) but not significant in males (although magnitude of change was similar in both groups)

(*continued*)

Table 1 (continued)

Citation	Phenotype/parameter	Male/females	Finding related to sex
Hoenicka et al. (2009)	Schizophrenia	226 cases, 117 controls/ 111 cases, 114 controls	Val^{158} homozygosity associated in men ($p = 0.022$) but not women
Katerberg et al. (2009)	OCD and patient phenotypic characteristics	151 cases, 235 controls/ 222 cases, 227 controls	Met^{158} associated with OCD in men ($p = 0.039$) but not women. Increased frequency of Met^{158} allele in women compared with men ($p = 0.012$). Sex × genotype interaction on OCD phenotype: heterozygous women showed a lower level of somatic and sensory symptoms compared with homozygotes ($p = 0.024$) but this relationship was absent in men
Kempton et al. (2009)	Brain activation during fearful affect processing	40/34	$Val^{158}Met$ modulates brain activation in regions differing between men and women. Sex × genotype interaction significant in right temporal pole ($p = 0.028$): female Val^{158} activate > Met^{158}, while male Met^{158} deactivate > Val^{158}
Quednow et al. (2009)	PPI[p]	54/53	Met^{158} associated with higher PPI in men ($p < 0.05$) but not women
Tsai et al. (2009)	Fluoxetine response in major depression	138/196	Val^{158} associated with poorer response in men ($p = 0.035$) but not women

[a]Findings all relate to $Val^{158}Met$ polymorphism except where stated
[b]Key studies, discussed in the text
[c]Haplotypes containing $Val^{158}Met$ were also studied
[d]VCFS: velo-cardio-facial syndrome (22q11 deletion syndrome)
[e]Calculated from their Table 1
[f]Elderly adults
[g]WMS-R: Wechsler Memory Scale (Revised)
[h]BNT: Boston Naming Test
[i]WCST: Wisconsin Card Sorting Test
[j]Representative number; exact sample size differed between tests
[k]Children
[l]*p* value not stated
[m]Weschler Intelligence Scale for Children
[n]Attention-deficit hyperactivity disorder
[o]Electroconvulsive therapy
[p]Prepulse inhibition

It should be noted that some phenotypic associations with COMT are in the opposite direction in the two sexes, rather than just being limited to a significant finding in one or other sex (e.g. Dauvilliers et al. 2001; Rybakowski et al. 2006). Results of this nature may be correct; however, in the absence of a prior hypothesis, or replication, they may well be false positives.

These confounds and limitations mean that most of the studies in the table are merely suggestive and do not by themselves provide convincing evidence that COMT genetic variation has a sexually dimorphic influence on the phenotype in question. Nevertheless, the number of studies showing at least some evidence for sexual dimorphisms is intriguing, especially as among the studies are several more striking and robust findings of this kind. We discuss these more robust findings further here.

The first study to report a sex difference in the role of COMT in the genetic predisposition to a psychiatric disorder (Karayiorgou et al. 1997) showed that the low activity (Met^{158}) allele was associated with OCD in men, but not in women in a case–control study. The authors later replicated this finding in a family-based study (Karayiorgou et al. 1999), and it was further replicated in three of four subsequent case–control studies (Poyurovksy et al. 2005; Denys et al. 2006; Pooley et al. 2007). This sex-selective association between the COMT Met^{158} allele and OCD was confirmed in a meta-analysis (Pooley et al. 2007); the odds ratio associated with the Met^{158} allele in men was 1.88 ($p < 0.001$), with no effect in women (odds ratio 0.98, $p = 0.83$), and with a significant sex difference between the odds ratios ($p < 0.0001$). Although studies published since this meta-analysis have produced mixed results (Wray et al. 2008 found no association between COMT in men or women, while Katerberg et al. 2009 showed a trend-level association between Met^{158} and OCD in men but not in women, in line with the finding of Pooley et al.), the COMT OCD data are arguably the clearest evidence to date for a sexually dimorphic autosomal genetic association with a psychiatric disorder.

Barnett et al. (2007a, 2009) also demonstrated a sexually dimorphic association between COMT and IQ. They genotyped initially the Val^{158}Met SNP (Barnett et al. 2007a), and more recently the functional haplotype described by Nackley et al. (2006) (Barnett et al. 2009), in over 5,000 participants in a longitudinal study of child development, and in whom a range of cognitive tests, including IQ, attention, and working memory, had been conducted between ages 8 and 10. In the initial study, the Met^{158} allele was associated with better function in several domains, with these effects greater in, or limited to, boys. More recently, they demonstrated that the strength of these associations was even greater when the functional haplotype described by Nackley et al. (2006) (which appears to have a larger effect on COMT enzyme activity than Val^{158}Met SNP alone) was considered. For example, in boys, Met^{158} homozygotes had a verbal IQ 3 points higher than Val^{158} homozygotes, whereas the difference in girls was less than 1 point. Strikingly, in the later study, the effect of the COMT haplotypes was even greater: boys carrying the COMT diplotype that predicted the highest enzyme activity had a verbal IQ 6 points lower, on average, when compared with boys with the diplotype predicting the lowest activity; again, there was no significant association between the COMT haplotypes

and verbal IQ in girls (Barnett et al. 2009). Intriguingly, in their initial study Barnett et al. (2007a) also showed that the COMT effect on verbal IQ was greater in pubertal than pre-pubertal boys (increasing to a 10 point difference between homozygote groups), suggesting that its influence increases with sexual maturation (they did not comment on the effect of age in their subsequent study). An increasing effect of COMT around the age of puberty is consistent with data linking COMT Val^{158}Met with verbal IQ in a longitudinal study of velocardiofacial syndrome (VCFS or 22q11 hemideletion syndrome, in which one copy of COMT is deleted; Gothelf et al. 2005). It is also consistent with significant maturational increases in COMT expression and activity in prefrontal cortex, although these changes were most marked post-adolescence and not peri-pubertally (Tunbridge et al. 2007a). Kates et al. (2006) also studied the role of COMT Val^{158}Met children with VCFS. Their finding, although in a small sample, is noteworthy because it concerns brain structure, and also because the dimorphism is statistically robust – a sex-by-genotype ANOVA interaction ($p < 0.001$), with an opposing effect of Val^{158}Met allele on frontal cortical volumes in boys and girls. Taken together, these data, together with those of Zinkstok et al. (2006), raise the possibility of a complex interaction between COMT genotype, sex, brain structure, and development.

Further evidence for sexually dimorphic associations between COMT and psychiatric phenotypes comes from hints that there may be sex differences in associations between COMT and schizophrenia. Shifman et al. (2002) reported that homozygosity for the G allele at rs165599 of COMT was strongly associated with schizophrenia in women ($p = 6.8 \times 10^{-6}$) but not in men ($p = 0.09$), with the sex difference in genotype effect being significant ($p < 0.01$). The authors concluded that there may be a sex-specific genetic component to schizophrenia, while acknowledging that twin studies had not predicted this. However, a sex difference in the genetic association with schizophrenia was not present for the other COMT SNPs they analysed, nor was it observed for haplotypes (that included rs165599). Furthermore, as noted by Craddock et al. (2006), their finding was driven by the sexually dimorphic allele frequency in the control group, described above. More recently, Talkowski et al. (2008) reported a female-specific association between COMT rs737865 and schizophrenia ($p = 0.008$) (but not in rs165599), in which the sex-by-genotype interaction was significant ($p = 0.0007$). They also replicated a female-specific association at this locus in a second cohort, although it was the other allele which was significantly associated in this second samples. Notably, this sex-specific association occurred in the absence of any sex differences in allele frequencies in the control group; thus, these findings are perhaps more convincing than those of Shifman and colleagues. However, despite the statistical significance of these two studies, the results remain difficult to interpret vis à vis schizophrenia, and in need of replication, especially given the different polymorphisms involved. The potential for sex differences in associations between COMT and schizophrenia is further complicated by the report of Sazci et al. (2004) of a female-predominant association (of Met^{158}) with schizophrenia, and by the fact that the meta-analyses of COMT Val^{158}Met with schizophrenia find no sex effect (Glatt et al. 2003; Fan et al. 2005). Thus, although these findings raise the intriguing

possibility that different loci within COMT may be differentially associated with schizophrenia in men vs. women, they should be considered preliminary and are in need of further investigation.

A further sex-by-genotype interaction ($p < 0.0005$) was seen by Lang et al. (2007) who showed that the Val^{158} allele was associated with the personality trait of sensation seeking in women but not in men. This finding is the most statistically robust of a line of studies that show relationships between COMT genotype and personality traits in women but not in men. Of particular note, anxiety-related phenotypes (such as harm avoidance and neuroticism) have been repeatedly (though often weakly) associated in women with the Met^{158} allele (Eley et al. 2003; Enoch et al. 2003; Olsson et al. 2005; Stein et al. 2005), consistent with findings in anxiety disorder (Domschke et al. 2004; Woo et al. 2004; Rothe et al. 2006) and with the increased anxiety found in female, but not in male, COMT knockout mice, described above. A meta-analysis of the panic disorder studies (Domschke et al. 2007) confirmed the presence of a sex difference in the association with COMT. However, this study also revealed an additional complexity: the relationship interacted with ethnicity, such that panic disorder was associated with the Met^{158} allele in Caucasian women but with the Val^{158} allele in Asian women. The latter result is consistent with the findings of Kim et al. (2006), who showed a Val^{158} association with harm avoidance in Korean women. Two main explanations come to mind for the genotype-by-ethnicity interaction. First, given that the resulting subgroups are quite small and the p values modest ($p = 0.04$ in Caucasians, $p = 0.02$ in Asians; Domschke et al. 2007), the findings are quite likely to be false positives. Second, if true, the finding may relate to ethnic differences in the genetic background of the Val^{158}Met polymorphism, such that the opposing allelic associations with panic disorder are both genuine (Lin et al. 2007). This possibility is quite plausible, given the complex manner in which SNPs in COMT have been shown to interact to regulate COMT activity (Nackley et al. 2006).

It is interesting to note that the data reviewed here suggest that the Met^{158} allele is associated with anxiety phenotypes in (Caucasian) women but with OCD – usually considered to be a type of anxiety disorder – in men. One interpretation is that Met^{158} is a risk factor for a shared predisposition to anxiety in both sexes, but that this manifests itself as different anxiety phenotypes in men and women because of other influences that are themselves sexually dimorphic, whether genetic, epigenetic, or environmental in origin.

4 Mechanisms Underlying Sex Differences in COMT's Effects on Brain Function and Its Associations with Psychiatric Disorders

Sexually dimorphic effects of COMT are normally explained by its regulation by oestrogens. Contemporary interest in oestrogenic COMT regulation can be traced to the work of Xie et al. (1999), although many years previously Axelrod had reported

that 17-β-oestradiol (E2) administration decreased COMT activity in rat liver (Cohn and Axelrod 1971). Xie et al. (1999) identified two oestrogen response elements in the COMT promoter and showed that E2 at physiological concentrations inhibits COMT mRNA expression in cells expressing oestrogen receptors, but not in those which do not. The same group later showed that the oestrogen-mediated decrease in COMT mRNA was accompanied by a proportional decrease in COMT immunoreactivity and activity (Jiang et al. 2003). This inhibitory regulation by oestrogens is consistent not only with the normal sex differences in COMT activity noted above, but also with the evidence that women with high oestrogen states (e.g. on the combined oral contraceptive, or in the third trimester of pregnancy) have lower COMT activity than other women (Briggs and Briggs 1973). In post-menopausal women, oestrogen levels fall dramatically, to substantially below that of men of the same age (Bjornerem et al. 2004); it would be of interest to know whether sex differences in COMT activity (and its genetic associations) present in younger adults are lost or reversed in the elderly.

In addition to itself being regulated by oestrogens, COMT in turn plays an important role in metabolising catechol oestrogens and thereby lowering levels of these potential carcinogens (Creveling 2003). There is both in vivo (Worda et al. 2003) and in vitro (Dawling et al. 2001; but see Goodman et al. 2002) evidence that the Val^{158}Met SNP influences this pathway with greater E2 metabolism in those with the high activity Val^{158} allele, a finding that may also explain the associations reported in some studies between the COMT Met^{158} allele and oestrogenic cancers (Goodman et al. 2002). These complex, reciprocal, and partly genotype-influenced interactions between COMT and oestrogens may be relevant to the question of sexual dimorphism. COMT genotype may modulate the role that oestrogens play in brain function and dysfunction (Seeman 1997), while oestrogens affect COMT activity and therefore its associations with behaviour and psychiatric disorders by virtue of their influence on COMT gene expression.

However, despite the focus on oestrogenic regulation of COMT gene expression, it is probably only a partial explanation for sex differences in COMT's function. Although COMT mRNA expression is lower in peripheral tissue in women (Wang et al. 2009), Cohn and Axelrod (1971) found that E2 did not affect rat brain COMT activity despite its robust down-regulation in the liver. Consistent with these findings, Jiang et al. (2003) demonstrated oestrogenic regulation of COMT in a breast cancer cell line but not in a glioblastoma cell line, again suggesting that this relationship may not pertain in the brain. The suggestion that oestrogenic regulation of COMT mRNA abundance may be less important for mediating COMT's sex differences in the brain, compared with the periphery, is supported by several recent human post-mortem brain studies showing that COMT activity is higher in men (Chen et al. 2004) even though COMT mRNA abundance is the same (Tunbridge et al. 2004b; Chen et al. 2004) or even higher (Dempster et al. 2006) in women. Hence, for a given level of COMT mRNA abundance, COMT activity in the male brain is higher than it is in females, and therefore the sexual dimorphism cannot be readily explained by transcriptional regulation. This fact also argues against potential explanations for sex differences in COMT based on epigenetic regulation of COMT mRNA (Kaminsky

et al. 2006); in any event, there is no evidence for a sex difference in COMT promoter methylation status (Abdolmaleky et al. 2006). Finally, the finding that COMT protein and activity levels rise considerably in men between the third and fifth decade of life (Tunbridge et al. 2007a), despite steady oestradiol levels across this period (Bjornerem et al. 2004) emphasises that oestrogens are not the only factor responsible for regulating COMT activity in the brain.

The mechanism mediating a sex difference in COMT activity in the absence of a difference in COMT mRNA and protein abundance is unknown. It is possible that the discrepancy is in fact spurious, since localised or phasic sex differences in COMT mRNA or protein abundance in brain – e.g. in specific cell types, or in relation to menstrual cycle – are likely, based on evidence from peripheral tissue (Salih et al. 2008). However, the fact that a robust sex difference in COMT enzyme activity was seen with no hint of a sex difference in COMT protein measured in the same samples (Chen et al. 2004) suggests that it is genuine. As such, one possibility is that it reflects a sex difference in the relative abundance of novel mRNA (Tunbridge et al. 2007b) or protein (Tunbridge et al. 2006b) isoforms of COMT, which could differ in their enzyme activity and might not affect the amount of total COMT mRNA or protein. Or, there could be a sex difference in the abundance of co-factors, endogenous inhibitors (e.g. *S*-adenosylmethionine [SAM]; Zhu 2002), or interacting proteins that modulate COMT activity. In support of the hypothesis that SAM levels may be rate-limiting in the female brain, there are hints from a small pilot study that SAM augmentation may be therapeutically beneficial for depressive symptoms for women but not for men (Strous et al. 2009)

More broadly, sexual dimorphisms in COMT occur against a background of sex differences in its biochemical pathways. In addition to differences between females and males in terms of catechol oestrogen function, the dopamine system is markedly sexually dimorphic (Di Paolo 1994; Becker 1999; Andersen and Teicher 2000; Carroll et al. 2004). For example, women have higher striatal [^{18}F]fluorodopa uptake, suggesting greater presynaptic dopamine synthesis (Laakso et al. 2002), and a lower D2 receptor affinity (Pohjalainen et al. 1998) than men, which the authors hypothesise reflects higher dopamine levels in the female brain at baseline. Conversely, women have lower amphetamine-stimulated dopamine release (Munro et al. 2006), and a greater dopamine transporter uptake, suggestive of more rapid clearance of dopamine from the synaptic cleft (Mozley et al. 2001). Thus, compared with men, women appear to have elevated basal, but decreased stimulated, striatal dopamine levels. Taken together, these findings may indicate a difference in the balance between tonic and phasic dopamine between women and men, a parameter which is also hypothesised to be regulated by COMT activity (Bilder et al. 2004). However, oestrogenic state (e.g. phase of menstrual cycle) has not been fully taken into account in these studies and may be a significant confounder: rodent studies have shown marked fluctuations in multiple dopaminergic parameters in the striatum across the oestrus cycle (Jori and Cecchetti 1973, Favis et al. 1977, Crowley et al. 1978; Fernandez-Ruiz et al. 1991; Morissette and Di Paolo 1993; Xiao and Becker 1994). Sex differences in dopamine function are less well studied outside the striatum; therefore little information is available to inform on relative

dopaminergic function in the sex differences in cortical regions where COMT is likely to have its primary impact (Karoum et al. 1994; Gogos et al. 1998; Tunbridge et al. 2004a; Yavich et al. 2007). Cortical tissue dopamine concentrations are reportedly similar in men and women (Robinson et al. 1977), although women may have a higher extra-striatal D2 receptor-binding potential (Kaasinen et al. 2001). In summary, there are diverse data suggesting substantial sex differences in central dopamine parameters (Cosgrove et al. 2007). However, it is not clear what their net effect is upon dopaminergic function in men compared to women, nor how the dopaminergic sexual dimorphisms impinge upon the inverted U-shaped relationship between dopamine activity and prefrontal function (Goldman-Rakic et al. 2000) known to be regulated by COMT activity (Mattay et al. 2003; Tunbridge et al. 2006a; Williams-Gray et al. 2007) and likely to be sensitive to interactive effects of COMT with other genes (Talkowski et al. 2008; Tan et al. 2007) and environmental factors (Caspi et al. 2005).

5 Conclusions

Genetic epidemiological and other studies show that sex differences in the genetic architecture of many human phenotypes, including psychiatric disorders, are common. There is also a substantial literature attesting to male–female differences across many domains of brain function, structure, and development. The data reviewed here suggest that COMT is one of the genes that contribute to these sexual dimorphisms. In addition to a difference in COMT enzyme activity between men and women, there is evidence that the involvement of COMT in predisposition to OCD and anxiety phenotypes is sex-specific, and weaker evidence for sex differences in its roles in several other phenotypes.

Not all COMT genotypic associations are demonstrably sexually dimorphic; for example, its influence on Wisconsin Card Sort Test performance (Barnett et al. 2007b) and on homocysteine metabolism (Tunbridge et al. 2008). Neither is COMT the only autosomal gene for which sexually dimorphic genetic associations with psychiatric phenotypes have been reported; using schizophrenia as an example, reelin is reportedly associated only in women (Shifman et al. 2008), while GNB1L (Williams et al. 2008) and MTHFR have been associated only in men (Sazci et al. 2005; Kempisty et al. 2006). However, the data do appear more extensive, and in places statistically more convincing, for COMT than for any other autosomal gene we could determine. Furthermore, as we have discussed, the findings parallel sex differences found in the COMT knockout mouse, and are complemented by the sexual dimorphism in COMT activity and by a plausible, if incomplete, mechanistic explanation in terms of oestrogenic regulation. As such, COMT may well contribute to the genetic basis for sexual dimorphisms in human brain, behaviour, and -related disorders, although it is clearly but one of many genes acting in this way, and in isolation explains only a tiny proportion of the variance. Future studies are needed not only to establish the range and magnitude of the COMT-related sexual

dimorphisms, but also to identify other genes, their epistatic and gene–environment interactions, and the underlying biological mechanisms.

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Sex Differences and Hormonal Influences in Human Sensorimotor Gating: Implications for Schizophrenia

Veena Kumari

Contents

Abstract Prepulse inhibition (PPI) of the startle response serves to prevent the interruption of ongoing perceptual and early sensory analysis and provides a simple operational measure of sensorimotor gating. In line with postulated deficits in early stages of information processing, PPI is disrupted in schizophrenia. PPI is considered a valid candidate for an endophenotypic marker in genetic studies of schizophrenia and has also been extensively used in translational research. Importantly, there are well-replicated sex differences and menstrual phase effects in prepulse-elicited startle modulation of nonclinical young populations. Lack of knowledge about the precise roles of sex differences and hormonal effects in prepulse-elicited startle modulation and in the schizophrenia disease process presents a stumbling block to continuous progress in this field. This chapter reviews a wealth of data demonstrating sex and hormonal influences in prepulse-elicited startle modulation and considers their implications for our understanding of the pathophysiology, genetics, and potential treatments of schizophrenia.

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1 Introduction

Schizophrenia is a severe neuropsychiatric disorder of unknown etiology. The condition is clinically heterogeneous and often results in disabling cognitive, perceptual, and emotional symptoms. The symptoms are generally classified as positive (e.g., hallucinations and delusions), negative (e.g., anhedonia, thought paucity), and cognitive (e.g., thought disorder, bizarre thinking). There are well-established sex differences in onset, prognosis, and course of this disorder (Hafner 2003). Current pharmacological treatments, largely involving blockade of dopamine D2 receptors (Kapur and Remington 2001; Guillin et al. 2007), are effective in reducing the acute symptoms but provide no cure. Accordingly, the need for a better understanding of the etiology and pathophysiology of schizophrenia and the development of novel treatments persists. The future of drug development in schizophrenia depends on many experimental strategies as well as on serendipity (Carpenter and Koenig 2008; Javitt et al. 2008). Over the last 30–40 years, translational models, such as prepulse inhibition (PPI) of the startle response, have played an important role in the strategic effort to develop and characterize new treatments. The aim of this chapter is to review sex differences and menstrual phase effects in PPI of nonclinical young populations and discuss their implications with a view to advance our understanding of the pathophysiology, genetics, and treatment of schizophrenia.

2 Prepulse-Elicited Startle Modulation (PESM) as a Measure of Sensorimotor Gating

The startle reflex consists of a set of reflexive, involuntary responses to a sudden, intense stimulus. It is known to exhibit several forms of plasticity which are remarkably similar in animals and humans, examples being habituation (Hoffman and Searle 1968) and fear potentiation (Brown et al. 1951). A further example of startle plasticity is that the startle response can be modified reliably by presenting a more innocuous stimulus (prepulse) before the strong startle-eliciting stimulus (pulse) (Graham 1975). When the time from prepulse onset to pulse onset, or stimulus onset asynchrony (SOA), is between 30 and 500 ms, such modification will appear as inhibition (PPI), evident as the attenuation of the startle response (Fig. 1). However, with a longer interval between the prepulse and the pulse, such modification will appear as facilitation of the startle response (PPF) (Fig. 1). PESM is increasingly being used as a measure of early and late information processing in experimental animals as well as in clinical and nonclinical human populations.

PPI is considered to provide an operational index of sensorimotor gating: while information processing resources are targeted at the prepulse, any incoming

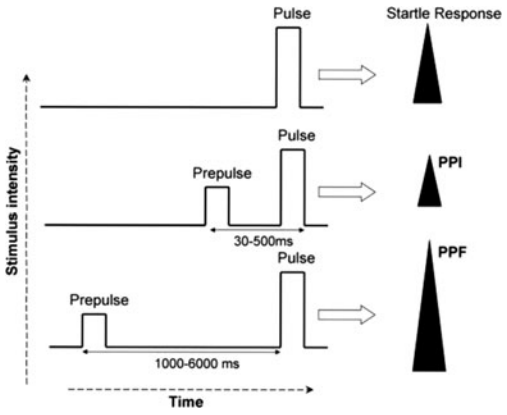

Fig. 1 PPI and PPF effects

information (i.e., the pulse) is attended to a reduced level, thereby protecting the processing of the initial stimulus (i.e., the prepulse). A reduced ability to gate (or filter out) such stimulus interference (i.e., reduced PPI) has been associated with sensory overstimulation and confusion (Geyer et al. 1990). Animal studies show that PPI is mediated by brain stem circuits involving the inferior colliculus, pedunculopontine tegmental nucleus, laterodorsal tegmental nucleus, substantia nigra pars reticulata, and caudal pontine reticular nucleus (Fendt et al. 2001) and modulated by forebrain circuits involving the prefrontal cortex, thalamus, hippocampus, amygdala, nucleus accumbens, striatum, ventral pallidum, globus pallidus, and subpallidal efferents to the pedunculopontine nucleus (Swerdlow et al. 2001, 2008). Although the neural substrates of PPI may vary somewhat between animals and humans (Swerdlow et al. 2008), imaging studies (Hazlett and Buchsbaum 2001; Kumari et al. 2003b, 2005a, 2007a; Postma et al. 2006; Hazlett et al. 2008) support the involvement of similar brain regions in modulation of human PPI. PPF is a relatively less well-studied phenomenon. It may reflect sustained attention (Dawson et al. 1997), sensory enhancement linked with modality (Anthony 1985), or a different aspect of the same mechanism underlying PPI (Kumari et al. 2003a). The neural substrates of PPF are not well studied even in animals. In the only study (Neuner et al. 2010) to have investigated the neural substrates of PPF (in healthy men only), many regions, consistent with previous PPI studies, were found to be activated for both PPI and PPF.

3 PESM Deficits in Schizophrenia

Graham (1975) suggested that "startle modulation might provide a powerful technique for probing what underlies the normal processing of information and especially for probing processing characteristics of relatively inaccessible subjects" (p. 238). Applying this to schizophrenia patients, Braff et al. (1978) demonstrated

impaired PPI confirming theorized deficits in early stages of information processing in this population. Many subsequent studies have replicated and refined this finding (Braff et al. 2001; Kumari et al. 2004, 2007b, 2008c; Swerdlow et al. 2006a; Takahashi et al. 2008). It has been hypothesized that a breakdown in the gating system causes sensory overload (Gottschalk et al. 1972) with secondary cognitive fragmentation, thought disorder, and possibly other psychotic symptoms in this clinical population (Braff and Geyer 1990). PPF is not as widely studied as PPI but is also impaired in schizophrenia, especially in female patients compared to female controls (Kumari et al. 2004).

PPI, given its high temporal stability (Abel et al. 1998; Cadenhead et al. 1999) and amenability to cross-species comparisons (Swerdlow et al. 1994), is one of the leading animal paradigms dominating research on antipsychotic drug activity and in other comparative psychopharmacological studies (Geyer et al. 2001; Swerdlow et al. 2006b; Talledo et al. 2009). Attesting the usefulness of PPI as a translational model, an emerging body of evidence suggests that second generation antipsychotics may attenuate the PPI deficit in schizophrenia (Kumari et al. 2000, 2007a; Swerdlow et al. 2006a).

PPI has also been recommended as a valid candidate for an endophenotypic marker in genetic studies of schizophrenia for several reasons. First, PPI is heritable with genetic factors contributing to 30–50% of the variance (Greenwood et al. 2007; Hasenkamp et al. 2010). Second, PPI is reduced not only in patients but also in schizophrenia-spectrum populations, for example, in unaffected biological relatives of schizophrenia patients (Cadenhead et al. 2000; Kumari et al. 2005b) and people with schizotypal personality disorder (Cadenhead et al. 2000) or high scores on psychometric measures of psychosis-proneness (Kumari et al. 1997, 2008b; Evans et al. 2005). Third, PPI is influenced by schizophrenia-relevant single nucleotide polymorphisms (SNPs) within the dopamine, serotonin, and acetylcholine systems (Quednow et al. 2008, 2009; Roussos et al. 2008a, b; Petrovsky et al. 2010). However, some of the genetically mediated effects in PPI found reliably in healthy men may not be present in healthy women who are tested without regard to their menstrual cycle phase. For example, catechol *O*-methyltransferase Val158Met SNP has been reported to significantly influence PPI in independent samples of healthy men (Roussos et al. 2008b; Quednow et al. 2009) but not of healthy women (Montag et al. 2008; Quednow et al. 2009). The most likely reason for this is menstrual cycle-related variability in PPI of healthy young women (next section). It is becoming increasing obvious that sex differences and hormonal influences need to be considered in future applications of PESM models in the context of schizophrenia and other disorders that are characterized by disrupted sensorimotor gating.

4 Sex Differences and Menstrual Phase Effects in PESM

PPI is sexually dimorphic (Fig. 2) with several studies reporting less PPI in healthy young women, when tested regardless of where they are in their menstrual cycle, than in healthy young men (Swerdlow et al. 1993, 1999; Abel et al. 1998;

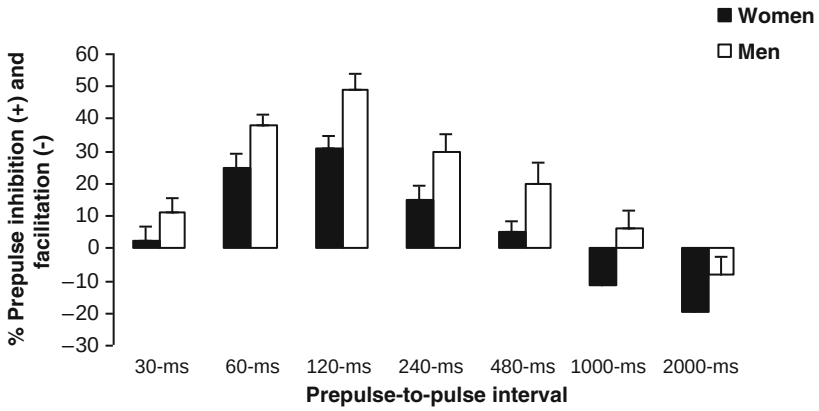

Fig. 2 Sex differences in PPI and PPF (data from Aasen et al. 2005). PPI and PPF = ([a − b])/a × 100, where "a" = amplitude over pulse-alone trials, and "b" = amplitude over prepulse trials (PPF is expressed as a negative value). PPI is seen to increase from 30-ms through 60-ms to 120-ms prepulse-to-pulse intervals, then to decrease and turn into PPF at 1,000-ms prepulse-to-pulse interval, especially in women. More PPI is present in men and more PPF in women

Kumari et al. 2003a, 2004, 2008a; Aasen et al. 2005). The sex difference in PPI of nonclinical young people remains true after possible confounds, such as cigarette smoking and personality, are controlled for (Swerdlow et al. 1999; Aasen et al. 2005). A sex effect in PPI (females < males) has also been reported in rats (Koch 1998; Faraday et al. 1999) and mice (Ison and Allen 2007).

PPI is sensitive to menstrual cycle status in healthy women, with more PPI observed during the early follicular phase relative to the luteal phase in both cross-sectional (Swerdlow et al. 1997) and within-subjects studies (Jovanovic et al. 2004; Kumari et al. 2010). Healthy pregnant women in their third trimester, when the levels of both estrogen and progesterone are roughly 50 times and 10 times (respectively) the levels in normally cycling women, have lower PPI in comparison with healthy postpartum women (Kask et al. 2008). Luteal phase young women show lower PPI in comparison with postmenopausal women (Bannbers et al. 2010). The sex effect in PPI is not present in children under 8 years of age (Ornitz et al. 1991), in postmenopausal women compared to age-matched men (Kumari et al. 2008a) or aged mice (Ison and Allen 2007).

Sex differences in PPF are less widely studied. Previous studies from our laboratory suggest that healthy young menstruating women display higher PPF than men (Kumari et al. 2003a, 2004, 2008a; Aasen et al. 2005) (Fig. 2) and greater PPF during the luteal relative to the early follicular phase (Kumari et al. 2010) (Fig. 3).

5 Hormonal Influences in PESM

Lower PPI during the luteal phase, compared to the early follicular phase, in healthy young women has been proposed to be caused by high levels of the ovarian hormone, estrogen (Swerdlow et al. 1997; Jovanovic et al. 2004). PPI is also

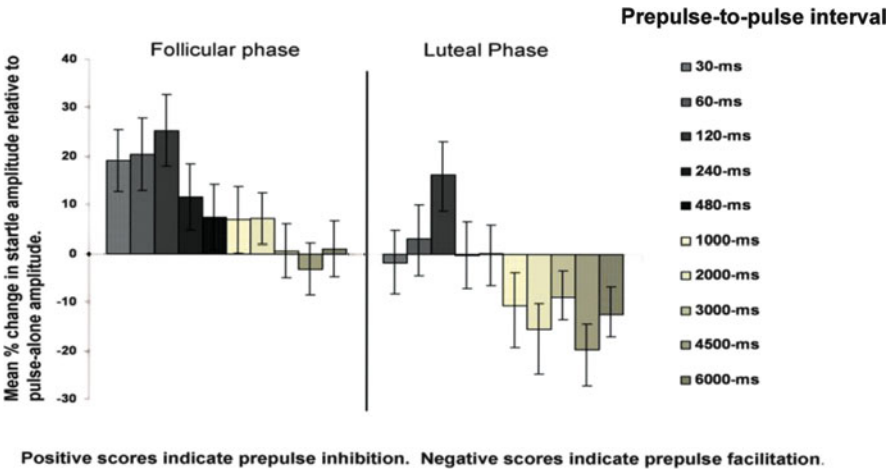

Fig. 3 Menstrual phase effects in PPI and PPF (data from Kumari et al. 2010). PPI increases from 30-ms through 60-ms to 120-ms prepulse intervals during both the follicular and the luteal phases, then decreases and turns into PPF at 1,000-ms especially during the luteal phase. Significantly more PPI is seen during the follicular phase and significantly more PPF during the luteal phase

reduced during periods of high estrogen in experimental animals (Vaillancourt et al. 2002). Estrogen influences various neurochemical activities including dopaminergic activity in the nucleus accumbens, an area critical for PPI both in experimental animals (Swerdlow et al. 2001) and in humans (Kumari et al. 2003b, 2005a). Administration of dopamine agonists, such as amphetamine or apomorphine, disrupts PPI in both rodents and human participants (Geyer et al. 2001; Swerdlow et al. 2003). Estradiol administration to ovariectomized rats has been reported to induce a decrease in the number of inhibitory synaptic inputs, an increase in the number of excitatory synapses, and an enhancement of the frequency of neuronal firing (Parducz et al. 2002). Since these findings fit the observed pattern of sex differences in PPI and PPF, we earlier suggested (Aasen et al. 2005) that women might show reduced PPI during the high estrogen phase of the menstrual cycle because estrogen elevates excitatory neuronal firing at the gate, inhibiting the gate to close as efficiently as men at short prepulse-to-pulse intervals and express increased PPF since the gate opens more efficiently at the long prepulse-to-pulse intervals compared to men. However, recent studies from our (Kumari et al. 2008a, 2010) and other laboratories (Talledo et al. 2009) have failed to detect a direct relationship between PPI and varying estrogen levels in healthy women, while in an earlier study estrogen administration (2 mg) prevented the disruption of PPI by buspirone, the serotonin-1A (5-HT1A) receptor partial agonist, but had no influence on PPI when given on its own to early follicular healthy women (Gogos et al. 2006).

Perhaps progesterone, another ovarian hormone, plays a role in menstrual cycle-related variability in PPI as suggested by findings of our most recent study (Kumari et al. 2010). This study showed a smaller decrease in PPI from the follicular phase to the luteal phase in women who had a larger increase in progesterone.

Progesterone shows marked fluctuations over the menstrual cycle (Marshall 2001) and possesses psychotropic properties in addition to its role in reproductive endocrinology (Rupprecht 2003). It is known to modulate the release of dopamine (Dluzen and Ramirez 1990; Ramirez and Zheng 1996) with biphasic effects, initially increasing but ultimately decreasing basal and amphetamine-stimulated dopamine release (Dluzen and Ramirez 1984). In rodents, progesterone reduces amphetamine-induced stereotypy (Michanek and Meyerson 1982). It is also implicated in the modulation of PPI (Rupprecht et al. 1999; Gogos and Van den Buuse 2004) and reverses apomorphine-induced disruption of PPI in rodents (Rupprecht et al. 1999). In healthy women, estrogen enhances the response to stimulant drugs, but this effect is masked in the presence of progesterone in healthy women (Justice and de Wit 1999). PPI, however, is sensitive not only to dopaminergic but also to serotonergic, glutamatergic, and cholinergic systems (Geyer et al. 2001; Swerdlow et al. 2008). Progesterone too, in addition to dopamine, affects other neurotransmitter systems. It is known to act as a functional antagonist at 5-HT3 receptors (Wetzel et al. 1998) and to have a role in the control of nicotinic cholinergic receptors (Valera et al. 1992). At present, it is unclear which of these systems might be most pertinent to the effect of progesterone in PPI of healthy young females.

6 Consideration of Sex and Hormonal Influences in PSEM in the Context of Schizophrenia

The first issue deserving consideration is the roles of ovarian hormones in the schizophrenia disease process. A later age of illness onset, less severe forms of the illness, a superior response to antipsychotics, as well as better functional and social outcomes are reported for women than men with schizophrenia (Castle and Murray 1991; Faraone et al. 1994; Castle et al. 1995), supposedly due to a neuroprotective role of estrogen in women (Hafner et al. 1998; Kulkarni 2009). Female schizophrenia patients display greater symptom severity during the periods of low estrogen (e.g., postpartum) and lower symptom severity during the periods of high estrogen (e.g., pregnancy) (Riecher-Rossler et al. 1994). These observations showing "reduced symptoms" with "high estrogen" appear inconsistent with the earlier proposal (Swerdlow et al. 1997) that high estrogen during the luteal phase causes lower PPI in healthy women. It is, of course, possible that estrogen–PPI relationship follows an inverted U pattern with both very low and very high levels producing lower PPI or, as mentioned earlier, is modulated by another ovarian hormone, progesterone. Our recent finding did suggest a role for progesterone, more specifically an antipsychotic-like PPI-restorative action of progesterone, during the luteal phase in PPI of healthy young women (Kumari et al. 2010). Considering this finding in the context of schizophrenia, women are more susceptible to the onset of schizophrenia after menopause and during the postpartum period (Hafner et al. 1993). This effect can be attributed to a drop in progesterone levels

(Shulman and Tibbo 2005). Studies have also reported high progesterone levels in unmedicated chronic patients in response to metabolic stress (Breier and Buchanan 1992) but normal progesterone levels in medicated patients with early psychosis (Oades and Schepker 1994) as well medicated chronic schizophrenia patients (Taherianfard and Shariaty 2004). Progesterone has been proposed to act as an endogenous antipsychotic and serve to restore normal function during times of stress (Shulman and Tibbo 2005). Further support for this notion comes from the reports that the progesterone metabolite 3α, 5α-THP produces a behavioral profile similar to that of dopamine receptor antagonists by increasing GABAergic tone in rodents (Khisti et al. 1998, 2002). Other data in rodents show increases in cortical progesterone and/or 3α, 5α-THP concentrations with olanzapine (Marx et al. 2000, 2003) and clozapine (Barbaccia et al. 2001; Marx et al. 2003), but not with haloperidol (Barbaccia et al. 2001). Atypical antipsychotic-induced increases in progesterone have been suggested to contribute to clinical benefits of these drugs (Barbaccia 2004; Marx et al. 2006). Studies so far have been incapable of disentangling the effect of estrogen and progesterone in human PESM.

The second issue is whether the "normal" reduction in PPI of healthy young women during the luteal phase means the same as "deficient" PPI in schizophrenia and related populations. As suggested earlier (Kumari et al. 2003a), reduced PPI in healthy women during the luteal phase "may not be a simple reduction but rather a shift of the inhibition/facilitation curve in the direction of facilitation in women, relative to men." At present, very few studies have examined both PPI and PPF in schizophrenia patients and not much is known about the pharmacology and neuroanatomy of PPF even in animals. It is plausible that schizophrenia is associated not only with impaired PPI but also with a defect in mechanisms that underlie a smooth transition of PPI into PPF with increasing prepulse-to-pulse intervals in women during the luteal phase and appear to be modulated by ovarian hormones.

7 Conclusions and Directions for Future Research

Given the importance of PPI as an animal model of schizophrenia and known sex differences in prognosis and course of this order, it is vital to uncover the biological basis of sex differences and menstrual cycle-related variations in PESM. While rodent studies in this area provide valuable information for hypothesis development, extrapolating their findings to humans is challenging because neuroanatomically "the further forward one moves in the brain, the greater the anatomical and functional differences between rodents and humans" (Swerdlow et al. 2008). Due to poor understanding of sex and menstrual phase effects in human PESM, the majority of imaging and pharmacological studies so far have used mostly, if not exclusively, men. Their findings are unlikely to be applicable to healthy or ill women. Future studies investigating pharmacological and treatment effects using a prepulse modification paradigm in normal and clinical populations of both sexes would benefit from consideration of sex differences and menstrual phase effects and

assessments of both PPI and PPF. Future research should also aim to clarify the roles of varying ovarian hormones, especially estrogen and progesterone, in menstrual phase-related variability in PESM and establish whether menstrual phase-related variability in PPI and PPF is mediated by estrogen alone, progesterone alone, or via their interaction. The knowledge gained from such research will enable further studies examining the therapeutic potential of hormones for treatment as well as prevention (e.g., postpartum psychosis) for disorders characterized by disrupted sensorimotor gating and, wherever relevant, combining them with genetic profiles.

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Estrogens and Gonadal Function in Schizophrenia and Related Psychoses

Anita Riecher-Rössler and Jayashri Kulkarni

Contents

Abstract Recent research has increasingly pointed to the importance of estrogens and the hypothalamic–pituitary–gonadal axis in schizophrenia. Specifically, there is mounting evidence from clinical, epidemiological, and basic research that estradiol, the main component of estrogens, exerts protective effects in schizophrenia and related psychoses. Possible modes of action of this hormone in the brain have been suggested, and clinical intervention studies have reported the first positive results.

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Furthermore, there are an increasing number of reports on gonadal dysfunction and states of estrogen deficiency in women with schizophrenia. These findings could have important implications for clinicians and researchers alike.

Keywords Estradiol · Estrogens · Gonadal function · Psychoses · Schizophrenia

Abbreviations

ESR1	Endogen receptor-α
HERS	Heart and Estrogen/Progestin Replacement Study
HRT	Hormone replacement therapy
PANSS	Positive and negative syndrome scale
RNA	Ribonucleic acid
WHI	Women's Health Initiative Study
WHI-M	Women's Health Initiative Memory Study

1 Introduction

Recent research increasingly points to the importance of estrogens and the hypothalamic–pituitary–gonadal axis in schizophrenia and related psychoses.

On one hand, there are reports of gonadal dysfunction and states of estrogen deficiency in women with schizophrenia (the hypothesis of hypoestrogenism). On the other hand, there is mounting evidence from clinical as well as from epidemiological and basic research that estradiol, the main component of estrogens, exerts protective effects in schizophrenia and related psychoses (the estrogen protection hypothesis) (Riecher-Rössler and Häfner 1993).

2 Estrogens: A Protective Factor in Schizophrenia and Related Psychoses? (The "Estrogen Protection Hypothesis")

2.1 Historical Findings

As long ago as at the beginning of the last century, psychiatrists recognized the possible association between schizophrenia and estrogens (for review, see Riecher-Rössler and Häfner 1993). There are longstanding observations indicating an association between lowered estrogen blood levels and *acute* psychotic symptomatology. Early clinicians such as Kraepelin and Kretschmer described signs of *chronic* "hypoestrogenism" in women with schizophrenia.

Kraft-Ebing was among the first to describe women becoming psychotic before or during menstruation, i.e., when blood levels of estrogen are relatively low. Kraepelin even created a separate diagnostic category, labeled "menstrual psychosis". Kretschmer reported cases where the outbreak of schizophrenia and related psychoses had a temporal relationship with "surgery of ovaries, pregnancy, delivery, and puerperium". Finally, Manfred Bleuler noted that late-onset schizophrenia with onset after age 40 years was much more frequent in women than in men, a finding he attributed to the "loss of ovarian function" starting at around that age (for review, see Riecher-Rössler and Häfner 1993).

2.2 Basic Research Findings

Important findings from basic research were the identification of estrogen receptors in the limbic system of the brain, and the observation that the effects of estrogens in rodents are, in some respects, similar to those of antipsychotic medications. Furthermore, it was shown that estrogens can modulate the sensitivity and number of dopamine receptors. It was therefore hypothesized that estrogens exert their antipsychotic effects in a manner similar to that of traditional antipsychotic medications at least partly by blockade of dopaminergic transmission (Riecher-Rössler and Häfner 1993).

We now know that estrogens, especially 17-β-estradiol (the natural estrogen that is most active in the brain), have many other neuroprotective and psychoprotective effects. For example, they appear to improve cerebral blood flow and glucose metabolism, promote neuronal sprouting and myelination, enhance synaptic density and plasticity, facilitate neuronal connectivity, act as antioxidants, and inhibit neuronal cell death.

Estrogens have also been shown to exert profound effects on brain differentiation during development, particularly during late gestation and the early postnatal period, and are important in normal maintenance of brain function during aging (Cyr et al. 2002; Goldstein et al. 2002; Oesterlund 2002; Vedder and Behl 2005). In a well-controlled magnetic resonance imaging study, Goldstein et al. (2002) showed that normal patterns of sexual brain dimorphism (brain regions found to be structurally different in normal men and women) are disrupted in schizophrenia and related psychoses, especially in the cortex. Apart from later "activational" effects of circulating hormones (e.g., during puberty), those investigators suggested that these early "organizational" effects of gonadal hormones that occur during the developmental period (which is probably critical for at least some forms of schizophrenia and related psychoses) could be partly responsible for that finding.

The mechanisms of action of estrogens are now known not only to depend on the classical genomic pathway but also to involve nongenomic, rapid interactions, which explain the differing latency of effects. They clearly modulate the dopaminergic and other neurotransmitter systems that are believed to be relevant to schizophrenia and related psychoses, such as the serotonergic and glutamatergic

system, but also the noradrenergic and cholinergic system (for reviews, see Cyr et al. 2002; Oesterlund 2002; Garcia-Segura et al. 2001; McEwen 2002; Stahl 2001a, b). Recently it has even been suggested that 17-β-estradiol in the brain might rather be regarded as a neurotransmitter itself than as a hormone (Balthazart and Ball 2006).

There are at least two subtypes of estrogen receptors, namely estrogen receptor-α and estrogen receptor-β, which are transcribed from two distinct genes (Oesterlund 2002). Autopsy studies showed that estrogen receptor-α messenger RNA is expressed in discrete areas of the human brain such as the amygdala, hypothalamus, cerebral cortex, and hippocampus; these areas are associated with neuroendocrine function, as well as emotion, memory, and cognition (Oesterlund et al. 2000).

Recently Weickert et al. (2008) reported a variation in the endogen receptor-α (ESR1) gene to be associated with schizophrenia and speculated that the mechanism of this association may involve alternative gene regulation and transcript processing.

Regarding the therapeutic effect of estrogens, it must also be noted that both the numerous direct effects on the brain and indirect effects may play a role. For example, estrogens may also increase blood levels of antipsychotic drugs via their actions on liver metabolism (Yonkers et al. 1992).

2.3 Epidemiological Findings

Epidemiological studies into sex differences in schizophrenic disorders suggest that the physiologically high estradiol production in young fertile women contributes to the later age of onset of schizophrenia in women as compared with men, to the second peak of onsets in women around the menopause, and to the better course of the disease in young women (Häfner et al. 1993; Riecher-Rössler et al. 1997). Thus, in an *epidemiological study* on a representative sample of 392 first admitted patients with schizophrenia, the ABC Study, we found that schizophrenic women have a later peak of illness onset in comparison with schizophrenic men (Häfner et al. 1991a, b; Riecher et al. 1991). They also exhibit an additional, smaller peak after age 45. We postulated that estrogens raise the vulnerability threshold for the outbreak of the disease. According to this hypothesis, women would be protected against schizophrenia between puberty and menopause to some extent by their relatively high gonadal estrogen production during this time. Then, around age 45, several years before menopause sets in at a mean age of 51.4 years, estrogen production begins to fall (Labhart 1978). Thus, women would lose the protection estrogens give, which could account for their second peak of illness onset after age 45.

A number of risk factors appear to counteract the protective effect of estrogens. Thus, the sex difference in the age of onset diminishes in the subgroup of cases with a genetic risk and in patients with perinatal complications (Häfner 2005; Könnecke et al. 2000).

Recent results regarding the age of menarche further support the hypothesis that physiological estrogens play a protective role against the development of the

disease. We demonstrated a significantly later age of menarche in a representative group of first admitted women with schizophrenia and related psychoses as compared with a healthy control group (Riecher-Rössler 2002). Seeman and co-workers (Cohen et al. 1999; Hayeems and Seeman 2005) found that later menarche was associated with an earlier onset of the illness, an association that was independent of factors such as family history and obstetric complications.

2.4 Clinical Findings

Clinically, psychotic symptomatology has often been found to correlate with the estrogenic state of women (for review, see Riecher-Rössler and Häfner 1993; Seeman 1996). For example, during high estrogen phases such as pregnancy, chronic psychoses appear to improve, whereas there is an excess of psychoses after delivery.

Psychosis associated with estrogen withdrawal due to conditions other than the puerperium was recently reviewed by Mahe and Dumaine (2001). Those investigators reported cases of premenstrual psychosis, post abortion psychosis, and psychoses associated with removal of hydatiform mole, cessation of oral contraceptives, clomiphene and tamoxifen administration (both estrogen receptor antagonists), and gonadorelin agonist administration (which blocks pituitary stimulation of endogenous estrogen secretion). Psychotic episodes were acute, short, and with a wide range of psychotic, but also affective, symptomatology. Recurrences were often reported when estrogen levels were normalized, and puerperal psychosis was frequent in the history of patients who were affected.

Psychotic symptoms in schizophrenic patients have also often been shown to deteriorate premenstrually or perimenstrually (i.e., in the low estrogen phase of the cycle; for review, see Riecher-Rössler and Häfner 1993; Riecher-Rössler 2002; Seeman 1996). Thus, Riecher-Rössler et al. (1994a, b) could show an inverse correlation of estradiol blood levels with psychopathology. They examined 32 acutely admitted women with schizophrenia, who gave a history of regular menstrual cycles, and found a significant excess of admissions during the perimenstrual low estrogen phase of the cycle ($p < 0.005$). During the hospital stay of the 32 women, there emerged a significant association between estradiol levels on one hand, and psychopathology scores on the other hand. Psychopathology seemed to improve when estradiol blood levels rose and vice versa. This was true not only for the total score of the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham 1962) and for almost all the subscores of this scale such as anergia, thought disturbance, activation, and hostile suspiciousness, but also for the general behavior on ward as rated by the nurses (NOSIE; Honigfeld et al. 1976) and for general well-being and paranoid feelings as rated by the patients themselves (BfS and paranoid subscore of PDS, both by von Zerssen and Koeller 1976).

Also, Bergemann et al. (2007a) found a significant effect of the menstrual cycle phase and 17-β-estradiol levels on positive and negative symptoms of 125 women

with schizophrenia and related psychoses. The same authors (Bergemann et al. 2008) could furthermore show a significant effect of estrogen on the comprehension of metaphoric speech and/or concretism, a main feature of schizophrenic thought and language disturbance. Ko et al. (2006) similarly found that 35 women with chronic schizophrenia and related psychoses had low levels of estrogen which was associated with severe negative symptomatology and reduced cognitive functioning, especially in the domains of verbal performance and executive tasks.

Most studies, however, did not examine the correlation between psychosis symptomatology and estradiol serum levels directly. For example, a more recent study (Choi et al. 2001) reported behavioral, affective, and somatic symptoms of schizophrenia (not psychotic ones) to be associated with the menstrual cycle phase. Estradiol was not measured.

Rather than examining cyclic fluctuations, Hoff et al. (1996) assessed the relationship between average estrogen levels from four consecutive weeks sampling with psychopathology and cognitive functioning in 22 female inpatients (aged between 22 and 63 years) with chronic schizophrenia. There was no significant association between average estrogen levels and psychopathology, but higher average estrogen levels were strongly associated with better cognitive abilities. However, this finding may be due in part to the effects of aging.

It has to be noted that an elevated number of admissions during the perimenstrual period has also been identified in other disorders (Althaus et al. 2000), and exacerbation of many psychiatric symptoms (not only psychotic ones) during the perimenstrual period was observed in patients with schizophrenia and related psychoses (Riecher-Rössler et al. 1994b; Harris 1997). In theory, this lack of specificity is to be expected because of the multiple effects of estrogen on mental functioning.

Studies on gender differences conducted in the area of late-onset schizophrenia emphasize the significance of hormone changes related to the menopause. Riecher-Rössler and colleagues (Riecher-Rössler et al. 1997; Riecher-Rössler 2002) showed that there are twice as many women as men with the onset of schizophrenia and related psychoses beyond 40 years of age, and that female patients with late-onset illness suffered from unexpectedly severe disease in terms of symptomatology and course. One explanation for this could be the fluctuation and sharp decline in estrogen levels just before and during the menopause. In support of this proposed explanation are results from long-term studies in women with schizophrenia showing that the course of illness in women tends to deteriorate rapidly during menopause and thereafter (for review, see Riecher-Rössler and Häfner 1993; Riecher-Rössler et al. 1998).

2.5 Intervention Studies

Intervention studies have also been conducted over long periods, mainly with positive results (Korhonen et al. 1995; Lindamer et al. 1997) (for review, see Riecher-Rössler and Häfner 1993). Thus, as early as the 1940s, Manfred Bleuler (Bleuler 1943) reported the first unsystematic trials using a combination of ovarian

and pituitary hormones. Mall (1960), a German psychiatrist in charge of a large hospital, examined 167 women suffering from schizophrenia with respect to estrogen excretion in 24-h urine samples, basal temperature, and vaginal cytology. Based on his findings, he divided the psychoses into two groups: hypofollicular and hyperfollicular. In the former group, he replaced estrogens and found that "hypofollicular psychosis can be healed relatively easily by this substitution therapy." Unfortunately, Mall does not give many details about these interesting studies.

In the first systematic trial conducted in 1996, Kulkarni et al. (1996) found that women with schizophrenia receiving estradiol as an adjunct to antipsychotic medication treatment exhibited rapid and greater improvement in psychotic symptoms than women receiving antipsychotics only. In 2001, the same group performed a double-blind, 28-day, placebo-controlled study (Kulkarni et al. 2001) in which 12 women were administered transdermal 17-β-estradiol (patches) 50 μg/24 h, another 12 women received 100 μg/24 h patches, and the third group received placebo patches. The 100 μg group experienced greater improvement than either the 50 μg or placebo groups, with striking improvements observed in the key psychotic symptoms.

Akhondzadeh et al. (2003) published a randomized study of 32 women of childbearing age with chronic schizophrenia. They administered ethinyl estradiol as an adjunct to haloperidol over 8 weeks and the control group received haloperidol only. The combination with estradiol showed a significant superiority over haloperidol alone regarding positive and negative symptom response. Furthermore, the estrogen group needed significantly less adjunctive anticholinergic medication to treat extrapyramidal side effects caused by haloperidol treatment. This finding is in line with other studies suggesting that estrogen treatment can also reduce the severity of antipsychotic medication-induced extrapyramidal side effects (Thompson et al. 2000).

Louza et al. (2004) did not find a positive response to estrogen treatment in their study, which they correctly discussed as possibly being due to having used conjugated estrogens rather than 17-β-estradiol. In a Cochrane review in 2005, Chua et al. (2005) surveyed data from only five randomized double-blind intervention studies with appropriate methodology. They concluded that the effects of estrogen as sole treatment or adjunctive therapy for those with schizophrenia and related psychoses were still unclear, but that further, larger clinical trials were needed. Studies in this review with negative results used conjugated estrogens and not 17-β-estradiol, although the latter has been shown to be the estrogen type with the most potent activity in the brain. Furthermore, to prevent endometrial hyperplasia, the estrogens were usually combined with progestogens which can counteract the positive effects of estradiol in the brain.

Kulkarni (2009) recently conducted a proof-of-concept study of 102 women with DSM-IV schizophrenia. In this double-blind randomized controlled 28-day study, women received either an active 100 μg estradiol skin patch treatment ($n = 56$) or an identical placebo patch ($n = 46$). All patients received antipsychotic drug treatment according to a standardized protocol. Progesterone was not given during the study; hence, the trial measured the impact of unopposed estradiol.

Psychopathology was assessed using the PANSS rating scale. Serum levels of estrogen, progesterone, prolactin, luteinizing hormone, and follicle-stimulating hormone were measured. Several cognitive tests were also administered. They found that patients who received the 100 μg estradiol adjunct made a significantly better recovery in their total positive, negative, and general symptoms of schizophrenia than the patients who received standard antipsychotic medication only ($p < 0.01$). Women who received the estradiol patch also showed significant improvement in cognition ($p < 0.01$). By measuring luteinizing hormone, they could also demonstrate that there is a direct effect on the pituitary gland, which suggests that this dose and type of unconjugated estrogen directly affects the hypothalamic–pituitary–gonadal axis.

Overall, these studies provide strong evidence for the estrogen protection hypothesis. The addition of transdermally delivered estradiol seems to be associated with significant abatement of psychotic symptoms in women with schizophrenia compared with standardized antipsychotic drug treatment alone.

Most of the estrogen treatment studies conducted so far have been in young, reproductive age women and not in peri- or postmenopausal women with estrogen deficiency. Theoretically, the greatest effect of estradiol would be expected when it is replaced in a woman in a hypoestrogenic state. Good et al. (1999) conducted a study in *postmenopausal* patients. He administered estradiol and progesterone to 14 women with schizophrenia, schizophreniform disorder, or schizoaffective disorder and found a significant improvement of negative symptoms over 6 months.

There are also some case reports regarding positive results of hormone replacement therapy (HRT) in postmenopausal women with schizophrenia and related psychoses. Bergemann et al. (2007b) reported a case study of a woman with first onset of schizophrenia in the perimenopause period. The patient experienced severe acute psychosis symptoms over several months, but refused antipsychotic treatment. As she was diagnosed to be in a periclimacteric state based on clinical symptoms and hormone analysis, she was started on transdermal estradiol in combination with norethisterone acetate and had an impressive remission of the psychotic symptoms. Lindamer et al. (1997) reported details about a postmenopausal woman, whose psychotic symptoms improved with estradiol treatment as an adjunct to her antipsychotic.

Lindamer et al. (2001) studied a community sample of postmenopausal women with schizophrenia and related psychoses. Twenty-four women received standard HRT, and 28 women had never received hormone treatment. Interestingly, the users of HRT needed a relatively lower average dose of antipsychotic medication and suffered fewer severe negative symptoms.

Ahokas et al. (2000) described positive effects of estrogen treatment in women with postpartum psychosis. In those women who exhibited sustained estrogen deficiency states, the addition of 17-β-estradiol, without any further medication, yielded a dramatic antipsychotic effect within 1 week. However, the proportion of schizophrenia-like psychoses in the sample was not given.

Finally, Kulkarni (2005) also tested the use of adjunctive estradiol in a small sample of 11 men with schizophrenia. They gave 2 mg oral estradiol valerate as an

adjunct to six men who were taking antipsychotic drugs. Five men received oral placebo plus their standard antipsychotic medication for 7 days. This small study was only conducted for 7 days to avoid feminization and other side effects in the men. The groups were matched for age, illness severity, and duration. Oral, rather than transdermal, estradiol was used to ensure treatment adherence in men with acute psychosis. Psychopathology was assessed using the standardized rating scales, PANSS and the BPRS, Brief Psychiatric Rating Scale. By day 5, the estradiol group showed significant abatement of psychotic symptoms compared with the placebo group, and by day 7, the estradiol group made further improvements. This study, although small in sample size and short in duration, raises the possibility that nonfeminizing estrogen may provide useful treatment possibilities for men with schizophrenia.

3 Hypoestrogenism in Women with Schizophrenia (The Hypothesis of Hypoestrogenism)

Several studies have recently confirmed earlier findings of disturbed gonadal function and hypoestrogenism in women with schizophrenia (Riecher-Rössler and Häfner 1993; Riecher-Rössler et al. 1994b, 1998; Choi et al. 2001; Kulkarni et al. 1996; Bergemann et al. 2002; Canuso et al. 2002; Hoff et al. 2001; Huber et al. 2001; Smith et al. 2002; Zhang-Wong and Seeman 2002). They described menstrual irregularities and reduced blood levels of estradiol, progesterone, and gonadotropins (follicle-stimulating hormone, luteinizing hormone) throughout the menstrual cycle, plus anovulation in the majority of women with schizophrenia and related psychoses. Reduced fertility was also reported.

There appear to be multiple reasons for these disturbances including the consequences of emotional stress and/or antipsychotic medication-induced hyperprolactinaemia, which is known to suppress gonadal function (Maguire 2002). However, these are probably not the only causes, because women experiencing other psychiatric disorders with similar emotional stress do not have the same hypothalamic–pituitary–gonadal axis hormone changes, at least not to the same degree (Riecher-Rössler et al. 1998; Huber et al. 2001). Furthermore, hypoestrogenism was observed long before the introduction of antipsychotics.

Smith et al. (2002) found the dose of typical antipsychotics to correlate with prolactin levels especially in women and prolactin to correlate inversely with estradiol serum levels. In contrast to those findings, Huber et al. (2001) were unable to identify a significant association of prolactin and estradiol in 43 women with acute psychosis, 14 women with other diagnoses, and 9 healthy control women. Nevertheless, the women with schizophrenia and related psychoses had significantly lower estradiol serum levels than the control women. Women with other psychiatric diagnoses fell in between the psychotic and the healthy group with regard to estradiol and prolactin levels. Also, Canuso et al. (2002) found a high rate of ovarian dysfunction and estradiol levels below normal, irrespective of

medication type or prolactin status in 16 premenopausal women with schizophrenia and schizoaffective disorders. Interestingly, Warner et al. (2001) found prolactin levels in unmedicated schizophrenic patients to be even lower than in control individuals. Those investigators suggested that this was due to a disordered dopaminergic system because dopamine tonically inhibits prolactin.

Taken together, these results imply that the hypothalamic–pituitary–gonadal axis is disturbed in many women with schizophrenia and related psychoses, and that the reasons for this are far from clear yet. An interesting research question in this context is whether gonadal dysfunction with estrogen deficiency could even be part of the underlying pathogenetic process, at least in a subgroup of women (Riecher-Rössler 2002).

4 Implications for Clinicians and Researchers

Further research into the impact of gonadal function and estrogen on schizophrenia and related psychoses is warranted because new diagnostic and therapeutic strategies could emerge that would benefit the many women worldwide who suffer from this disorder.

4.1 Assessment and Therapy of Gonadal Dysfunction

As there is growing evidence that many, even younger women, with schizophrenia and related psychoses are in a state of estrogen deficiency, in future estrogens and the gonadal axis should be considered more seriously in the treatment of women with schizophrenia and related psychoses. Psychiatric history taking should always include questions regarding menstrual irregularities, amenorrhoea, and galactorrhoea. Also, prolactin and estrogen serum levels should be tested, if necessary. Gonadal dysfunction and hypoestrogenic states can often be found even in menstruating women (Riecher-Rössler et al. 1994b, 1998; Smith et al. 2002). In addition, hyperprolactinaemia is clearly underdiagnosed (Maguire 2002). Some authors have therefore suggested routine laboratory tests (Smith et al. 2002).

Most antipsychotics can cause hyperprolactinaemia and – especially if they are taken over a number of years – theoretically induce “iatrogenic early menopause” via suppression of physiological estradiol production. The concomitant risks include both short-term effects, such as hot flushes and sexual dysfunction, and long-term consequences, including osteoporosis and potentially cardiovascular disease or cognitive deterioration (Oesterlund 2002; Maguire 2002). In schizophrenia patients, these risks are further increased by additional risk factors such as smoking, poor diet, and reduced exercise (Smith et al. 2002). Furthermore, menopausal complaints may lead to compliance problems.

In the case of hyperprolactinaemia with secondary estrogen deficiency, prolactin-sparing antipsychotics (e.g., clozapine, quetiapine, aripiprazole, or maybe olanzapine;

Maguire 2002) should therefore be preferred. If a switch to these antipsychotics is not possible for clinical reasons or if hypoestrogenism persists despite switching, then estrogen can be added to the treatment. Issues regarding contraception must be taken into account in such cases because, when switching to prolactin-sparing antipsychotics, the menstrual cycle often normalizes and fertility is regained, with high risk for unplanned pregnancy (Neumann and Frasch 2001).

4.2 Estradiol as a Therapeutic Agent?

First trials of estrogens in schizophrenia and related psychoses indicate that estradiol could be used as an adjunct to antipsychotic medication. However, further replications of these findings in larger control studies by different groups are needed before recommendations for broad clinical application can be made.

In women who suffer from frequent perimenstrual psychotic relapses, "cycle modulated" antipsychotic medication therapy or, if contraception is needed at the same time, continuous use of oral contraceptives without hormone-free intervals may be strategies worthy of research (Riecher-Rössler 2002; Braendle et al. 2001).

Even more promising could be hormonal replacement with estrogens in women with schizophrenia in peri- and postmenopause, because estrogens in other disorders such as depression have proven to be especially helpful when they are used to restore hormonal balance.

Hormonal replacement with estrogens for women with schizophrenia *during and after the perimenopause* could be recommended as an augmentation strategy respectively an adjunct to antipsychotic medication. Possibly, the dose of antipsychotics could then be reduced and corresponding side effects minimized. The replacement of estrogens in these women could also attenuate perimenopausal complaints such as hot flushes, night sweats with sleep disturbances, and general irritability (see Table 1), which can contribute to a general deterioration of the mental state and, in vulnerable women, potentially provoke a psychotic episode. In contrast to this recommendation, it has been reported that women with schizophrenia are less likely to ever use HRT as compared to women without psychiatric diagnoses (Lindamer et al. 2003).

Estrogen replacement therapy for women of this age group has been recommended anyway for many reasons, for example prophylaxis of osteoporosis, and also delay of age-dependent cognitive deterioration or Alzheimer's dementia (Sherwin 2005) (for review, see Riecher-Rössler and de Geyter 2007; Table 1). Further research into estrogen treatment as an *additional* indication in peri- and postmeno-pausal women schizophrenia and related psychoses is urgently needed.

The use of estrogen has been questioned in the context of perimenopausal estrogen replacement by studies such as the WHI, Women's Health Initiative Study (Rossouw et al. 2002), the WHI-M, Women's Health Initiative Memory Study (Craig et al. 2005), and the HERS, Heart and Estrogen/Progestin Replacement Study (Hlatky et al. 2002). These studies have highlighted the side effects of

Table 1 Some important effects of estrogen replacement

Positive	Negative
Perimenopausal complaints ↓	Endometrial carcinoma ↑ if unopposed estrogens are administered (→ in women without hysterectomy always combine with progestogens!)
Physical: hot flushes, genital discomfort, aging of collagen (skin, joints, intervertebral discs) ↓	
Mental: depression, irritability, emotional lability ↓	
Risk of osteoporosis ↓	Risk of breast cancer ↑? (→ do not use in patients with a familiar or own risk of breast cancer and usually not longer than 7 years!)
Delay of cognitive decline/Morbus Alzheimer?	Risk of thrombosis and cerebral insult ↑? (→ no prescription for patients at risk!)
Cardiovascular protection? (if started right after menopause)	Other cardiovascular risks (coronary heart disease, arteriosclerosis) ↑? (→ start only within the first 10 years after menopause and not in patients with cardiovascular disease!)

Sources: Riecher-Rössler and de Geyter (2007), Birkhäuser et al. (2008), Rossouw et al. (2007)

HRT, which has provoked an ongoing controversy about the advantages and disadvantages of this regime.

However, the WHI study has been criticized by many experts and by the International Menopause Society (Birkhäuser et al. 2008) because of the advanced age of the study population (mean age at inclusion was 63 years) who had a high prevalence of cardiovascular risk factors. Many of the complications noted for participants in the WHI study such as stroke, pulmonary embolism, and myocardial infarction, which were attributed to the vascular effects of estrogens, could well have been due to pre-existing arteriosclerosis.

The WHI conclusions have now been partially counteracted by a reanalysis (Rossouw et al. 2007), which showed that the cardiovascular complications can be reduced using replacement therapy early in the perimenopause. This has opened a window of opportunity in which possibly even a cardiovascular benefit can be obtained in healthy menopausal women when replacement therapy is started early after the menopause (for review, see Riecher-Rössler and de Geyter 2007).

Overall, the use of estrogens for *therapeutic* reasons must be distinguished from their *preventative* use. The WHI investigated the latter use and as described above, concerns about the conclusions have been made. The potential *therapeutic* use in women with psychosis or other mental disorders has been discussed here, and while care must be given to monitoring potential side effects, the benefits for women with poor quality of life due to intractable schizophrenia often outweigh the risk of side effects.

Further research needs to be done into the best mode of HRT for psychiatric patients. To date, the natural 17-β-estradiol has been shown to have the best neuropsychoprotective effects compared with other estrogens that are often prescribed. Transdermal application in the form of patches or gel is preferred because of smoother metabolism and potentially fewer side effects. Progestogens are usually added to estrogens to prevent endometrial cancer, but they can antagonize the

positive effects of estrogens with respect to mental state (Cyr et al. 2002; Braendle et al. 2001). These systemic effects of progestogens should be minimized by careful selection of the progestogen, and also, potentially by other forms of application.

Furthermore, alternatives to conventional HRT, i.e., compounds with more specific and potent estrogenic activity in the brain as opposed to other tissues, need to be investigated (Riecher-Rössler 2002; Halbreich 2002). Such compounds would both minimize the side effects of hormonal therapy and permit new therapeutic strategies in men. Possible candidates are selective estrogen receptor modulators, which have agonistic or antagonistic properties that depend on the target tissue. However, the effects of the available selective estrogen receptor modulators on the brain remain to be clarified. Raloxifene, for example, appears to exert its main effects on the bone, although recent data suggest that it also acts on different brain receptors (Craig et al. 2005). A recent study by Kulkarni et al. has shown promising antipsychotic effects for adjunctive raloxifene in postmenopausal women with schizophrenia (Kulkarni et al. 2010). Also, the synthetic steroid tibolone appears to cause less endometrial proliferation, but its effects on the central nervous system are still not clear, apart from the fact that it appears to have an androgenic effect and increases β-endorphin levels, with improvement in mood and libido (Davis 2002). Further studies on the brain-specific effects of selective estrogen receptor modulators and other estrogenic compounds (e.g., phyto-estrogens, xeno-estrogens, and dihydroepiandrosterone) are urgently needed.

5 Summary and Conclusions

In summary, there is emerging evidence that estrogens are very useful neuroprotective and psychoprotective adjunctive therapies, which could complement and enhance the traditional drug therapies for people with schizophrenia and related psychoses. However, it must be emphasized that the role for estrogen treatment strategies still requires further research. In particular, results from larger, well-controlled studies are needed before estrogens may be recommended as adjunct therapy in standard clinical practice for the treatment of women with schizophrenia, without proven estrogen deficiency.

In contrast, other strategies should already be part of standard clinical care (Grigoriadis and Seeman 2002). These include examination of the gonadal axis, with therapeutic actions, if indicated. In peri- and postmenopause HRT can be used, but the decision to use estrogen replacement therapy must always be made on the basis of an individual risk–benefit assessment (Brikhäuser et al. 2008) and in close collaboration with a gynecologist.

For future research, many questions remain unresolved, regarding not only new therapeutic strategies and compounds but also the poorly understood disturbances of estrogens and the hypothalamic–pituitary–gonadal axis in women with schizophrenia and related psychoses. Further research in this area may substantially contribute to our understanding of the pathogenesis of this disease, at least in a subgroup of women.

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Oestradiol and Psychosis: Clinical Findings and Biological Mechanisms

Angelika Wieck

Contents

Abstract Female sex steroids easily access the central nervous system and modulate a number of intracerebral processes via their specific receptors. Oestradiol is the biologically dominant female sex steroid and has been implicated in the aetiology and course of psychotic illnesses. There is evidence for interaction between oestradiol and several neurobiological systems that have been implicated in the pathogenesis of psychotic illnesses. Clinical studies have indicated that psychosis, and in particular schizophrenia, is associated with reduced ovarian function and that this may be inherent to the illness itself. In schizophrenia several studies have suggested a therapeutic effect of oestradiol and selective oestrogen modulators although research is still at an early stage. In bipolar disorder, the relationship between childbirth and first onsets or recurrences is one of the most reproducible findings in psychiatric research. Whether or not the rapid fall of oestrogens is the mediating mechanism is not yet clear but preliminary oestrogen treatment studies commenced immediately after childbirth are promising. Outside the perinatal context, tamoxifen, a selective oestrogen receptor modulator, has

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shown strong antimanic effects although further studies are necessary to test an effect in larger samples. Hormonal treatments should not yet be used in standard care but could be considered in women with treatment resistant psychoses.

Keywords Bipolar · Mechanism · Oestrogen · Oestradiol · Psychosis · SERM · Schizophrenia · Treatment

1 Introduction

Research into the biological mechanisms that mediate gender differences in psychosis and the influence of reproductive events on the course of psychosis in women has focussed on female sex steroids, and particularly oestradiol as the predominant ovarian steroid. In this chapter, the relationships between oestradiol, ovarian function and the course of the two major psychoses, namely schizophrenia and bipolar disorder, the biological mechanisms that may be involved and recent treatment trials that have used oestrogens and oestrogen receptor modulators will be discussed.

2 Clinical Studies That Link Oestradiol with Schizophrenia

There are several lines of evidence that link oestrogen with the course and severity of schizophrenia. In epidemiological studies, the age of illness onset has consistently been shown to be 4–6 years later than in men (Lewine 1988; Häfner et al. 1991; Castle and Murray 1991). This applies not only to the first sign, first negative symptom, first positive symptoms, and first full episode, but also to the first admission (Häfner 2003). Women have also been reported to have a second peak of onset in the perimenopause (Häfner 2003).

Several recent studies have reported that female inpatients and outpatients with schizophrenia tend to have hormonal profiles consistent with insufficient maturation of ovarian follicles and anovulatory cycles, even during treatment with prolactin-sparing antipsychotic medication (Riecher-Roessler et al. 1994; Huber et al. 2001; Canuso et al. 2002; Bergemann et al. 2005). This indicates a hypothalamic–pituitary dysfunction or a reduced ovarian sensitivity to pituitary peptide stimulation. However, alternative explanations for an impaired ovarian activity such as weight gain (Brewer and Balen 2010) induced by psychotropic medication and stress (Genazzani et al. 2010) on ovarian activity have not been ruled out. Despite the low oestradiol levels in schizophrenia, their fluctuations have nevertheless been shown to influence symptom severity. Riecher-Roessler et al. (1994) investigated 32 women, who were admitted for the treatment of an acute psychotic exacerbation and were on antipsychotic medication. In the weeks subsequent to admission, measures of overall illness severity, thought disturbance and paranoia

inversely correlated with oestradiol levels. Furthermore, Seeman (1983) described that women ageing between 20 and 40 years require lower antipsychotic doses than older women or men and suggested that oestrogen may have anti-dopaminergic action. In line with this observation, Gattaz et al. (1994) showed a significant inverse association between oestradiol plasma concentrations and the required dose of antipsychotic medication.

Childbirth is the reproductive event that is associated with the largest changes in female sex steroid production. During pregnancy, oestradiol and progesterone serum concentrations gradually increase and reach values at term that are 100–200 times higher than in the early follicular phase of the menstrual cycle. Immediately following the expulsion of the placenta, levels begin to decline rapidly and reach follicular phase levels within 2–3 days. It has been known for some time that childbirth can trigger psychotic illnesses, but two recent epidemiological studies covering the whole population of Denmark over three decades have quantified this effect for different diagnoses more precisely (Munk-Olsen et al. 2006, 2009). In these studies, inpatient admissions for first onsets and recurrences of schizophrenia were 2–5.7 times more common in the 2 months after childbirth than later in the first postnatal year.

Based on these several strands of evidence, it has been suggested that oestrogen protects women from an early onset and severe course of illness, but that this advantage is lost in the perimenopause when oestrogen production declines (Häfner et al. 1989; Seeman and Lang 1990). These findings also suggest that women with schizophrenia may benefit from treatment with oestradiol to enhance this effect and that the dose required may not be large.

3 Clinical Studies That Link Oestradiol with Bipolar Disorder

There are no findings of a gender difference in the age of onset in bipolar disorder. However, one of the most remarkable findings in psychiatry is the powerful relationship of this illness with childbirth. In the studies by Munk-Olsen et al. (2006, 2009), the risk of a first or subsequent inpatient admission for bipolar disorder was increased more than 20 times in the first month after childbirth. Based on the dopamine hypothesis of bipolar disorder, Cookson (1982) suggested that bipolar episodes in the puerperium may be triggered by the rapid and massive decline of oestrogen or progesterone after childbirth via its effect on the dopaminergic system (Cookson 1982).

Whether the fall of sex steroid concentrations at the end of the menstrual cycle is associated with a worsening of bipolar symptoms is controversial. Two early retrospective studies found greater affective symptomatology in the premenstrual phase in women with bipolar disorder than healthy controls (Diamond et al. 1976; Price and DiMarzio 1986). However, in a cross-sectional interview study of women with recurrent major depressive disorder ($N = 509$) and bipolar 1 disorder ($N = 197$), Payne et al. (2007) found significant associations between histories of

postpartum affective symptoms and premenstrual or perimenopausal mood changes in the major depression group (odds ratios 1.82 and 1.66) but not in the bipolar group. Karadag et al. (2004) prospectively followed 34 stable medicated patients with bipolar disorder and 35 healthy controls. Participants kept daily records of their mental and physical wellbeing over 2 months and completed an interview on their experiences across menstrual cycles. The bipolar group complained less often about mood changes, such as mood lability, depressive symptoms, and anger and irritability than the control group. In two prospective studies of rapid cycling bipolar patients, there were also no significant effects of the premenstrual cycle phase on bipolar mood changes (Wehr et al. 1988; Leibenluft et al. 1999).

There are two reasons why these studies are difficult to interpret. First, bipolar patients in these studies were on mood stabilizing medication, which may have suppressed premenstrual affective symptoms. Second, several authors commented on the high rate of menstrual cycle abnormalities in the bipolar subjects, and this may mean that their endocrine states across menstrual cycles were different from the controls. As in women with schizophrenia, several explanations need to be considered for such ovarian dysfunction. These include antipsychotic-induced hyperprolactinaemia (Wieck and Haddad 2003), valproate-induced polycystic ovarian syndrome (Joffe et al. 2006) or a dysregulation of the hypothalamic–pituitary– ovarian axis inherent in bipolar disorder. Rasgon et al. (2005) found that half of their 80 female study participants with bipolar disorder reported on interview that they had menstrual irregularities before commencing anti-bipolar medication. Similarly, in the Harvard Study of Moods and Cycles, Joffe et al. (2006) found that significantly more women with bipolar disorder reported early-onset menstrual cycle dysfunction (34.2%, before the onset of their illness) than healthy controls (21.7%).

Brockington (2005) reviewed the world literature on bipolar-like psychoses that repeatedly recur at the same phase of the menstrual cycle. However, this phenomenon has mostly been described in case reports or case series and does not appear to occur commonly.

4 Oestradiol in the Brain

About 1–3% of the total plasma oestrogen and progesterone circulate in serum unbound to proteins (Wu et al. 1976; Darne et al. 1987; Meulenberg and Hofman 1989) and are free to enter the brain by diffusion. The concentration of total oestradiol and progesterone in CSF correlates strongly and significantly with that in plasma (Bäckström et al. 1976), and the free fraction in plasma is of about the same magnitude as the total hormone in cerebrospinal fluid (Schwarz and Pohl 1992). On the other hand, CSF levels of the sex steroid-binding proteins (albumin, steroid hormone-binding globulin and cortisol-binding globulin) are several hundred times lower than in serum, suggesting that most of the total oestrogen and progesterone in CSF are free (Schwarz and Pohl 1992).

Oestrogens have a broad spectrum of actions in the central nervous system, which are mediated either via rapid alteration in signal transduction via membrane receptors or by slower modulation of gene transcription via receptors located intracellularly (Marshall 2011). Two oestrogen receptor subtypes, alpha and beta, have been identified, and their genes are located on different chromosomes. In the human forebrain, both subtypes are predominantly expressed in limbic-related areas although their distribution patterns differ (see review by Hughes et al. 2009). The mRNA expression of the alpha receptor appears to dominate in the hypothalamus and amygdala, areas concerned with autonomic and reproductive neuroendocrine functions as well as emotion interpretation and processing, whereas the beta isoform is dominant in the hippocampal formation, the entorhinal cortex and the thalamus, suggesting a possible role in cognition, non-emotional memory and motor functions (Hughes et al. 2009).

5 Mechanisms That May Mediate Oestradiol Effects in Schizophrenia

Oestrogen effects on the function of the mesolimbic and mesocortical dopaminergic and hippocampal glutamatergic systems are particularly relevant to psychoses. It is clear that dopaminergic neurones have oestrogen receptors (Creutz and Kritzer 2002), and that oestrogen interacts with dopaminergic systems (Sánchez et al. 2010). However, there is substantial variability in the direction of effects, reflecting differences in dose, duration of treatment, experimental protocols and outcomes studied (Chavez et al. 2010; Sánchez et al. 2010).

An experimental tool that has been widely used in the exploration of neurobiological mechanisms in schizophrenia is prepulse inhibition (PPI); see chapter by Veena Kumari (2011) for more details. This is the reduction of a startle response to a sudden loud noise if it is preceded by a weak prepulse within 30–500 ms. The inhibition of this reflex is modulated by brain circuits linking the limbic cortex, striatum, pallidum and pontine tegmentum and higher brain structures, such as the prefrontal cortex and hippocampus (Swerdlow et al. 1997), so that sensory information can be filtered and attention focussed. PPI is reduced in schizophrenia, some other psychiatric and several neurological disorders (Braff et al. 2001). A reduction in PPI during treatment with dopamine agonists and a reversal by antipsychotic agents in rodents suggest a contribution of the dopamine system in the modulation of PPI (Zhang et al. 2007; Mansbach et al. 1988) In schizophrenic patients, the PPI deficit may also be reversed by atypical antipsychotic treatment (Wynn et al. 2007; Aggernaes et al. 2010). Furthermore, recent studies suggest that dopaminergic modulation of PPI can be influenced by the hormonal milieu. For example, in female ovariectomized rats, high dose oestrogen treatment over 2 weeks has been shown to prevent apomorphine-induced disruptions of PPI, and that this may be mediated via an action on dopamine D2 receptors (Gogos et al. 2010). In healthy women, variations in sex steroid levels are also accompanied by changes in PPI. A greater PPI has been observed during the

follicular phase relative to the luteal phase of the menstrual cycle (Swerdlow et al. 1997; Jovanovic et al. 2004). However, Kumari et al. (2010) did not find a correlation between changes in PPI and oestradiol levels between menstrual cycle phases but reported that a greater progesterone increase in the luteal phase was associated with a greater protection of PPI. The relationships between physiological changes in female steroid production, dopaminergic function and PPI have not yet been investigated in human females with or without schizophrenia.

However, the usefulness of PPI as a model for neural processes operating in schizophrenia is limited, since PPI decreases are neither specific for schizophrenia nor do they predict the pattern of symptoms, the course of illness or individual treatment responses (Swerdlow et al. 2008).

Although research of neurotransmitter function in schizophrenia focussed for many years on excess dopaminergic function in the striatum, more recently it has been suggested that this is secondary to dysfunctional glutamatergic neurotransmission (Carlsson et al. 2001), a hypothesis that has recently been confirmed by Stone et al. (2010). In this imaging study of drug-free subjects at very high risk of schizophrenia, the authors found a negative correlation between glutamate levels in the hippocampus and striatal dopaminergic activity. Preliminary evidence indicates that oestradiol can modulate glutamatergic function in the hippocampus and other brain regions (Smejkalova and Woolley 2010; Grove-Strawser et al. 2010). Whether this interaction is relevant to schizophrenia requires further research in preclinical and human studies.

6 Mechanisms That May Mediate Oestradiol Effects in Bipolar Disorder

Although effective treatments are available for bipolar disorder, a comprehensive pathophysiological model of the illness is still lacking (Cousins et al. 2009). In their review of the role of dopamine in bipolar disorder, Cousins et al. (2009) conclude that this neurotransmitter system is likely to play a central role in the understanding of the pathophysiology of this illness. They also suggested that it may be the second messenger systems and downstream pathways that are directly involved rather than presynaptic processes or postsynaptic dopamine receptors. Overactivity of one element in the dopamine signal transduction pathway, protein kinase C, has been associated with acute mania and several known antimanic agents, such as lithium, valproate, carbamazepine, aripiprazole and quetiapine, inhibit it (reviews by Zarate and Manji 2009; Cousins et al. 2009). Recent studies have also shown that the selective oestrogen receptor modulator tamoxifen is a centrally active protein kinase C inhibitor and has therefore been tested as an antimanic agent.

There are to date only two biological studies in humans that investigated biological mechanisms that might be involved in the triggering effect of childbirth on bipolar recurrences. To test the hypothesis that they are triggered by the

effects of oestrogen withdrawal on the dopaminergic system, the apomorphine-induced growth hormone response was used as a measure of neurotransmission via hypothalamic D2 receptors. In the first study (Wieck et al. 1991), 15 drug-free women who had a history of bipolar illness but were currently well and 15 control women with no psychiatric history were recruited in late pregnancy. The neuroendocrine test was carried out on day 4 after delivery, and those who subsequently had a recurrence had a significantly higher response than those who remained well. However, the stimulation of hypothalamic growth hormone secretion is relatively blunted in the early postnatal period, and the study may have underestimated differences in the neuroendocrine response between the women who relapsed and those who did not. In the second study, hypothalamic D2 receptor sensitivity was tested across the menstrual cycle in eight medication-free well women with a history of puerperal bipolar illness and normal menstrual cycles and nine normally menstruating controls (Wieck et al. 2003). Subjects underwent the same apomorphine growth hormone test in the early follicular phase when oestrogen levels are low and in the midluteal phase when oestradiol levels are enhanced. The midluteal time point was defined as 7–11 days after the pre-ovulatory LH surge which was determined by ovulation test kits. Although the women with bipolar disorder were currently well, they had an enhanced response to apomorphine in the midluteal phase. This indicates that the hypothalamic dopaminergic system of women predisposed to puerperal bipolar illness responds more sensitively to changes in oestradiol levels. In view of recent findings, it is possible that this hypersensitivity is related to a dysfunction in the intracellular signalling system rather than the D2 receptors themselves. It is uncertain, however, whether such hypothalamic effects are representative of regulatory processes in the mesolimbic or mesocortical dopamine systems.

7 Treatment Studies in Schizophrenia

Chua et al. (2005) conducted a systematic review of oestrogen treatment trials in schizophrenia or related non-affective psychoses. They identified five randomized placebo-controlled studies including a total of 122 patients. Oestrogen treatments were adjunctive to antipsychotic therapy and lasted between 3 weeks and 6 months. No significant effect of active treatment was found on psychopathology. However, the authors concluded that the existing literature was difficult to interpret on account of the small sample sizes, randomization issues and the differences between studies in respect of menopausal status, phase of illness, type of oestrogen used, routes of administration and dose. A large double-blind randomized placebo-controlled trial ($N = 102$) of transdermal oestradiol treatment (100 μg over 24 h) over 28 days as adjunct to antipsychotic medication was published by Kulkarni et al. (2008). Participants were inpatients or outpatients, had a diagnosis of schizophrenia, schizophreniform disorder or schizoaffective depression and were in the acute or chronic phase of illness. At baseline, the mean score on the Positive and

Negative Symptoms Scale (PANSS) was similar to that reported by the authors of the scale for their sample of inpatients with schizophrenia (Kay et al. 1989). There were no differences between the groups in regard to adverse oestrogen-related or motor side effects. However, there were significant improvements in the active treatment group for the total PANSS score ($p < 0.002$), the positive symptom subscale ($p < 0.005$) and the general psychopathology subscale ($p < 0.01$), but not the negative symptom subscale. This pattern of response is often seen in trials of antipsychotic medication.

The transdermal application of 17-β oestradiol has the advantage of avoiding first pass liver metabolism. However, oestrogen treatment has several drawbacks. Its long-term effects in premenopausal women is not known, and in postmenopausal women it increases the risk of endometrial and breast cancer as well as myocardial infarct and stroke. Raloxifene is a promising alternative to 17-β oestradiol since it is an antagonist at the breast and does not promote endometrial cancer. However, it has been associated with a small increase in the risk of venous thromboembolism (Nelson et al. 2009). While the amounts entering the brain are reported to be small, pharmacological CNS effects have nevertheless been reported in animals (Littleton-Kearney et al. 2002) as well as in postmenopausal women (Neele et al. 2001).

Treatment with raloxifene was piloted in a dose-finding randomized controlled trial by Kulkarni et al. (2010) in 35 postmenopausal women in the acute phase of schizophreniform or schizoaffective disorder. Participants allocated to either 60 or 120 mg of raloxifene or placebo as adjuncts to antipsychotic medication over 12 weeks. Despite the small sample size, at the end of treatment there was a significant decrease in the total PANSS score ($p < 0.001$) and the general symptom score ($p < 0.02$) in the group treated with the larger dose.

8 Treatment Studies in Bipolar Disorder

Despite the high risk of bipolar recurrences in the immediate postpartum period, only few investigators have attempted testing the preventative or acute efficacy of pharmacological interventions. Due to ethical constraints, randomized controlled studies cannot be conducted in late pregnancy and large-scale observational naturalistic studies are logistically difficult, due to the relatively low number of patients at risk in the catchment areas of individual hospitals. Such studies require the collaboration of specialists in perinatal psychiatry from several centres.

Two open uncontrolled studies have prospectively tested the effect of oestrogen treatment commenced after delivery. The rationale was to soften the impact of the rapid decline of oestrogen levels following the expulsion of the placenta. Sichel et al. (1995) administered conjugated equine oestrogens to 11 drug-free women with histories of predominantly puerperal psychosis which is thought to be closely related to bipolar disorder. Oestrogens were administered immediately

after childbirth at a high dose (eight times the maximum postmenopausal dose) with heparin protection, and the dose was gradually stepped down. Only one woman relapsed. This contrasts with the reported rates of postnatal recurrences of 26–57% in groups of women with a history of bipolar/schizoaffective disorder irrespective of their medication status (Reich and Winokur 1970; Dean et al. 1989; Robling et al. 2000; Jones and Craddock 2001; Robertson et al. 2005) and rates of 40% and 70%, respectively, in two medication-free samples (Marks et al. 1991; Viguera et al. 2000). In a dose-finding study of 29 drug-free women with histories of bipolar or schizoaffective disorder, transdermal patch treatment delivering 200, 400 and 800 μg oestradiol per 24 h was commenced within 48 h under heparin protection and stepped down over 12 days (Kumar et al. 2003). All women were admitted for at least the duration of the trial. The recurrence rate was not lower than expected (41%), but the highest starting dose (equivalent to 16 times the postmenopausal dose) was associated with a significantly shorter duration of admission than the two lower doses. It is possible that the difference between the two studies is due to the timing of the first dose. In the second study, treatment may have been initiated too late since the decline in oestradiol and progesterone is immediate and rapid after the expulsion of the placenta. However, due to the concern over postpartum thrombosis as well as postpartum bleeding during protective heparin treatment, the risks of commencing treatment immediately after childbirth could be too high to pursue this approach further.

In an open uncontrolled trial, ten women with established and severe puerperal psychosis were treated with oestradiol for 6 weeks (Ahokas et al. 2000). The term "puerperal psychosis" is often used in clinical practice to denote the proximity to childbirth without specifying the type of psychosis. Because a large proportion of these illnesses are on the bipolar spectrum, the study was included here. Participants had an illness onset on day 12 on average and entered the study at a mean of 12 weeks postpartum. Four patients had been treated unsuccessfully with antipsychotic medication before the trial. Oestradiol was administered sublingually to avoid first pass metabolism by the liver. None of the women had menstruated since delivery, and oestradiol levels were very low at baseline. The daily oestradiol dose was titrated according to serum concentrations with the aim of reaching concentrations of 400 pmol/L, i.e., about one-third of the peak level during the regular menstrual cycle. The score of the Brief Psychiatric Rating Scale fell dramatically within the first week from 78.3 to 18.8 ($p < 0.001$), and by week 2 the patients had become almost symptom-free. One woman discontinued oestradiol by week 5 and had a full recurrence of symptoms in week 6.

Recently, several studies have tested the antimanic properties of the selective oestrogen receptor modulator tamoxifen in male and female patients with bipolar disorder. In a single blind study, tamoxifen up to 80 mg daily lead to a rapid improvement in five of seven patients with mania (Bebchuk et al. 2000). In another small, 4-week, double-blind, placebo-controlled, add-on study, treatment with 40 mg tamoxifen ($n = 5$), medroxyprogesterone acetate ($n = 4$) and placebo ($n = 4$) was compared (Kulkarni et al. 2006). Subjects in the tamoxifen group had a significantly greater decrease in manic and positive psychotic symptoms

compared to the placebo group ($p < 0.05$). All patients were receiving concomitant medication with either lithium or valproate. Similar results were obtained in two subsequent 3-week double-blind placebo-controlled, monotherapy studies. The study by Zarate et al. (2007) tested higher doses of up to 140 mg/day in a sample of 16 patients, and ratings were obtained daily during the first week. Significant improvements were seen as early as day 5, and the difference to placebo remained significant up to the end of treatment ($p < 0.001$) with a large effect size ($d = 1.08$). In the largest study ($N = 66$), Yildiz et al. (2008) used doses of up to 80 mg/day over 3 weeks. Significant improvement in the ratings of mania and clinical global impression (both $p < 0.001$) was reported at the end of treatment. In a fifth study (Amrollahi et al. 2011), tamoxifen or placebo were randomly added to treatment with lithium in 40 patients with acute mania. A significantly greater improvement was apparent at week 1 in the active treatment group, and this difference continued until the end of treatment in week 6.

The sample sizes in these trials are small, but the results are consistent and suggest that tamoxifen may be an effective antimanic agent that is well tolerated and acts rapidly. Drawbacks of the treatment are an increased risk of thromboembolic events and endometrial cancer. It may therefore be more suitable for short-term treatment of severe or treatment-resistant acute mania, particularly in men.

Although tamoxifen belongs to the group of selective oestradiol receptor modulators, it is thought that it involves a direct action on protein kinase C rather than the oestrogen receptor (O'Brian et al. 1986).

9 Conclusion

Insights into the various aspects of the relationship between oestradiol and psychotic illness are still limited. Although there is considerable evidence for a role of oestradiol, particularly for schizophrenia, not all available research supports this.

Nevertheless, in a recent well-designed randomized controlled treatment trial, women with acute schizophrenia-like illnesses responded well to the use of transdermal oestradiol (Kulkarni et al. 2008). The oestradiol dose of 100 μg/24 h is relatively low for a premenopausal patient group, since it only achieves levels that are similar to the early follicular phase of the menstrual cycle (Chetkowski et al. 1986). The mechanism by which the improvement was mediated is also not clear as yet. At the beginning of treatment, the patients were in different endocrine states. About one-third of the patients were taking antipsychotic medication with prolactin-elevating potential, and others entered the study in different phases of the menstrual cycle. The pattern of oestradiol levels across the 4 weeks of treatment that resulted from the interactions of endogenous hormone production, transdermally applied hormone and its potential to disrupt ovulation is difficult to predict. The mean oestradiol serum levels were low at baseline before treatment was begun (71.0 pg/ml). Potential mechanisms that could explain a therapeutic effect include that oestradiol levels

stayed above a certain critical level or that the treatment led to less fluctuations over the treatment period than would have otherwise occurred. Further clinical trials by different research groups that examine pituitary gonadal peptide and female sex steroid secretion during treatment are required to test this promising treatment further. Preliminary data on the therapeutic efficacy of raloxifene are also encouraging, particularly because it has a less adverse side-effect profile. Further preclinical research is required as to its actions in the central nervous system.

In bipolar disorder, it is not clear whether there is a pre-existing immaturity in the hypothalamic–pituitary–ovarian axis, and future studies should clarify this important point. The strong relationship of bipolar disorder with childbirth is a much replicated finding, and the predominant hypothesis has been that the postpartum oestrogen withdrawal triggers a latent dopaminergic dysfunction. There is some indirect evidence to support this view. There is also uncontrolled evidence that oestradiol treatment may be useful in the treatment of puerperal bipolar or affective psychotic episodes although it may only be worth pursuing this treatment in established illness rather than in prevention due to the risks of oestradiol treatment immediately after childbirth. The potential of raloxifene or other new selective oestrogen receptor modulators in puerperal and nonpuerperal bipolar illness should be explored.

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Sex Differences Precipitating Anorexia Nervosa in Females: The Estrogen Paradox and a Novel Framework for Targeting Sex-Specific Neurocircuits and Behavior

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Contents

Abstract In anorexia nervosa (AN), *reward contamination* likely plays a significant role in maintenance of the illness. Reward contamination is a context in which patients' behaviors of self-starvation and excessive exercise, while initially

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rewarding, become aversive, *even punishing*; but patients may not recognize the punishing and conflicted/contaminated behaviors. An emerging neurocircuit encompassing the anterior cingulate cortex (ACC) has been functionally linked to symptoms including reward contamination and body dysmorphic processing. Owing to the significantly greater prevalence of AN in females, evidence from clinical literature and preclinical models is spearheaded to provide a novel rationale for estrogen triggering sensitivity to the experience of stress and reward, precipitating AN disproportionately in females at the time of puberty. Paradoxically, however, estrogen may facilitate response to pharmacological interventions and (desensitization of the identified neurocircuits) via its contribution to serotonin modulation, hypothalamo-pituitary adrenal (HPA)-axis attenuation, and effects on dopamine.

Keywords ACC · Antipsychotics · DLPFC · Dopamine · DRN · Estrogen · HPA-axis · Neurocircuits · OFC · Reward contamination · Sensitivity · Serotonin · SSRI

1 Introduction

Anorexia nervosa (AN) is a relatively uncommon illness (affecting up to 4.3% of women across their lifetime, Wade, et al. 2001) but it is predominantly diagnosed in adolescent females, in up to 95% of cases, (DSM-IV-TR 2000) with a prevalence of 0.3–1.3% (Hoek and van Hoeken 2003; Bulik et al. 2006; van Kuyck et al. 2009). Several rationales for the sex-specific predominance of females diagnosed with AN have been proposed; however, this chapter will focus on a novel role for estrogen contributing to the disproportionate precipitation of the illness.

Self-starvation and excessive exercise are core illness features (Davis 1997) which have been linked to *anhedonia* [the reduced capacity to experience *reward* (Keating 2009)], which is consistent with reports that more excessive exercisers tend to be more anhedonic (Davis and Woodside 2002). The diagnostic relevance of reward to AN remains to be established, albeit the contribution of *sensitivity or capacity for reward* among patients has been considered an important factor influencing the clinical expression of AN (Davis and Woodside 2002; Keating 2009) and its development (Keating 2009) including the contribution to *reward contamination*, which likely plays a significant role in the disorders' maintenance (Keating 2009). Reward contamination is a context in which patients' behaviors of self-starvation and excessive exercise, while initially rewarding, become aversive *or punishing*, but patients may not *recognize* the punishing (and contaminated) features of their behaviors. Contaminated reward processing has been proposed to be mediated by abnormalities in the anterior cingulate cortex (ACC) among a broader network (Keating 2009).

Diagnostic criteria for AN does not encompass reward abnormalities, but includes weight relative to height (A), intense fear of gaining weight or becoming

fat (B), body weight and shape distortions (C), and amenorrhea in postmenarcheal females (D) (DSM-IV-TR 2000), the latter demonstrating physiological dysfunction. With regard to amenorrhea, there are few differences in demographics, eating behaviors, body image perceptions, illness history, and psychiatric comorbidity distinguishing menstruating and nonmenstruating patients (Garfinkel et al. 1996; Abraham et al. 2005) with many questioning the relevance of this criterion to the illness (e.g., Roberto et al. 2008; Attia and Roberto 2009). Physiologically, elevated stress, hypothalamo-pituitary adrenal (HPA) axis activity is reported in the majority of patients with AN linked to their behaviors (Bergh and Sodersten 1996) and is logically consistent with hypothalamo-pituitary gonadal (HPG)-axis-suppression-induced amenorrhea.

This chapter will focus on a novel explanation for a role for estrogen (around puberty) facilitating illness onset disproportionately in females, via enhancing *sensitivity to stress and reward* associated with AN behaviors. In addition, a paradoxical role for estrogen in the neuropsychopharmacology of response to treatment in AN (Keating et al. 2010) will be developed, the latter owing to estrogen's relationship to serotonin modulation, HPA-axis modulation, and indirect evidence for an association with reward (dopamine) normalization, of which these systems have been putatively linked to AN.

1.1 Stress Induces Reward, and Both Are Linked to the Development of Anorexia Nervosa

Consistent with reports that AN behaviors are linked to anhedonia, the illness has been suggested to develop because it is initially rewarding to eat less food and AN behavior is maintained through conditioning to the situations that provide reward (Bergh and Sodersten 1996; Sodersten et al. 2006, 2008). At a pathophysiological level, the majority of patients present with HPA-axis hyperactivity (Bergh and Sodersten 1996; Keating et al. 2010; Licinio et al. 1996, among others), whereby elevated corticotropin-releasing hormone (CRH) levels, up to 170% of normal, have been linked to self-starvation (Bergh and Sodersten 1996). The physiological sequalae of CRH release is the stimulation and secretion of cortisol from the adrenal glands.

Adrenocortical hormones are rewarding because their secretion stimulates the release of dopamine (DA) from the ventral srtiatal terminals of the mesolimbic neurons in the brain via both adrenocortical hormone feedback on these terminals and activation of DA cell bodies in the ventral tegmentum of the mesencephalon (at least in an animal model of the illness, Bergh and Sodersten 1996). Importantly, adrenocortical secretions enhance the *reward value* of AN-linked behaviors by increasing the release of DA in the terminals of these neurons (Bergh and Sodersten 1996; Piazza et al. 1993), presenting a potently physiologically reinforcing mechanism for AN behaviors.

This explanation encompasses why patients experience their behaviors (excessive exercise and food restriction) rewarding in the first instance (Keating 2009) and has been extended psychologically and physiologically, to rationalize why patients continue engaging in these behaviors despite the fact that they are aversive, even punishing (Keating 2009).

1.2 In the Disorder Maintenance Phase, a Neurocircuit for Reward Contamination May Play a Significant Role in Persistent Poor Decision Making

There are several neurobiological explanations for development of AN; however, few consider the distinction between factors influencing onset relative to maintenance of the illness. This chapter will focus on stress-induced reward linked to the onset of AN, as well as the ultimate contamination of reward and punishment (Keating 2009). While behaviors linked to AN are initially rewarding and associated with illness onset, reward contamination likely plays a significant role in illness maintenance, due to the fact that patients may ultimately fail to recognize the punishing (or contaminated) features of their behaviors. Reward contamination has been proposed to be physiologically mediated by *overlapping* neural circuits responsible for processing *both* reward and punishment (Keating 2009) that become contaminated and reinforced, as a result of pathological and unrelenting engagement in disordered-linked behaviors.

A body of literature implicates dysfunction in reward processing in AN (reviewed in Keating 2009); however, no studies have systematically investigated reward contamination, that is, patients' perceptions of *punishment* associated with challenges where the *hedonistic* (reward) rating of tasks is assessed (i.e., testing whether a reward-based task is considered punishing, and vice versa, whether a punishing task is considered rewarding, Keating 2009). Recently, however, an investigation in individuals recovered from the illness may indirectly shed clinical light on reward contamination linked to AN.

During functional magnetic resonance imaging (fMRI), participants recovered from AN (and healthy controls) were required to process *reward* and *punishing* stimuli (e.g., wins and losses on a gambling task) with results demonstrating in recovered individuals that, neurally, the ventral striatum failed to distinguish reward from punishment, whereas healthy controls distinguished these stimuli in the same region (Wagner et al. 2007). While this study did not intend to investigate reward contamination per se, a failure to distinguish between rewarding and punishing stimuli in ventral striatum (the region of interest, ROI, in this study, Wagner et al. 2007) in recovered individuals supports the contention of reward contamination, given healthy controls were able to distinguish between these experiences in the same region.

Extrapolated to the reward contamination theory (Keating 2009), these results (from Wagner et al. 2007) support the ventral striatum as a reward-contaminated locus in recovered individuals. Moreover, the persistence of neural contamination within this region, following recovery from the illness, suggests that reward contamination in the ventral striatum (at least) reflects a vulnerability marker for the illness (Keating 2009), which is furthermore supported by data from an investigation in *ill* patients, similarly demonstrating dysfunction in this region, again linked to reward contamination, albeit not intending to test this.

For example, Fladung et al. (2010) investigated in ill AN patients, activity in the ventral striatum in relation to *self-referent* processing of pictures of women at different weights, including *underweight* and *normal weight*. Although Fladung et al. (2010) were interested in demonstrating reward-linked starvation dependence associated with the ventral striatum (Fladung et al. 2010; Keating 2010), their results may also infer important information regarding body dysmorphic self-perceptions and reward contamination linked to this region in AN (Keating 2010). An argument for interpreting the results by (Fladung et al. 2010) as an illustration of reward contamination is based on the fact that when processing pictures of women at different weights in a *self-referent manner*; patients demonstrated more *positive appraisals* correlated with activity in the ventral striatum to *underweight* relative to normal-weight pictures, reflecting an indirect illustration of reward-conflict given that it would *otherwise* be expected that an underweight picture may induce feelings of *aversion*, which is consistent with and supported by the fact that healthy control participants demonstrated the *opposite association*; e.g., more positive ratings of normal weight compared with underweight women also linked to activity in this region (Fladung et al. 2010).

The extent to which reward contamination may reflects a marker for AN in recovered individuals (e.g., interpretations based on data presented in Wagner et al. 2007) as well as symptoms of AN beyond body dysmorphic perceptions (Keating 2010) and neuroanatomically, whether the substrate for these psychological processes are diffuse within other brain regions, or the likelihood of a neurocircuit, remains to be directly tested.

1.2.1 An Emerging Neurocircuit for Anorexia Nervosa

Neuroanatomy

Regarding other regions involved in reward contamination, the ACC has been proposed as a key locus and nexus in reward contamination contributing to disorder maintenance in AN (Keating 2009). This rationale is consistent with both preclinical and clinical literature demonstrating its heterogeneous functions in reward (Petrovic et al. 2008; Kennerley et al. 2006) punishment (Wrase et al. 2007) and conflict (Pochon et al. 2008) processing (reviewed in detail in Keating 2009), as well as reward-punishment contamination demonstrated in another psychiatric illness, major depression (Knutson et al. 2008). Abnormalities in structure and

function of the ACC region are some of the most frequently cited in AN literature (reviewed in Keating 2009); however, there remains to be a consensus on a disorder-specific explanation for a role of the ACC in AN.

Empirical evidence for the functional contribution of the ACC to AN, linked to reward, punishment, and the *contamination* of these experiences has been provided by an analyses of data (Kaye et al. 2009) from recovered individuals (Wagner et al. 2007). Kaye et al. (2009) revealed data that enables an interpretation to support the assertion that recovered individuals fail to neurally distinguish reward and loss (punishment) extending from the ACC (hypoactivated in recovered individuals) through to the ventral striatum, providing further support for a neurocircuit (at least the ACC and ventral striatum) involved in reward contamination.

Although a region of interest analysis was not extended to the OFC and dorsolateral prefrontal cortex (DLPFC) among other executive regions, it is proposed too, these areas, owing to their links to decision making based on reward (and reward history) (e.g., Kerns et al. 2004; Kennerley et al. 2006) as well as abnormalities in set-shifting in AN (Zastrow et al. 2009) may also contribute to or enable, reinforcement of disorder linked poor (or contaminated) decision making and action (behavior) selection, for which these regions are putatively involved (e.g., Kerns et al. 2004; Kennerley et al. 2006; Rushworth et al. 2007). Further analysis of more executive regions in recovered individuals (Wagner et al. 2007), but also in ill patients, would reveal the extent to which a neurocircuit may contribute to reward contamination. Furthermore, in the illness state, whether patients "self-referent processing" of pictures of body images (or processing of their own body images) and their experience of *reward or punishment* (theoretically) linked to these appraisals, extends beyond the ventral striatum, and whether these abnormalities persist in recovered individuals may further reveal the contribution of reward contamination to this pervasive disorder–symptom, as well as presenting another functional biomarker for the illness which may be linked to relapse–risk.

Neurochemistry

The neurochemistry of AN is complex. Abnormalities present in several systems including the HPA-axis (e.g., Bergh and Sodersten 1996; Licinio et al. 1996), the dopaminergic (e.g., Frank et al. 2005), and the serotonergic systems (which has been extensively reviewed, e.g., Kaye 2008), with persistent abnormalities demonstrated in the latter two systems (e.g., Bosanac et al. 2005) following weight gain. Dysfunction in the DA system is commonly reported, including greater D2 and D3 receptor binding in the ventral striatum during positron emission tomography (PET) (at baseline) in patients recovered from the illness (Frank et al. 2005) as well as abnormalities (overactivity) of the DA system in patients (e.g., Barbato et al. 2006). Functionally, DA has also been linked to processing both *reward and punishment* (Matsumoto and Hikosaka 2009) (in an animal model). Taken together with the fact that AN is linked to abnormalities in the DA system in the ventral striatum (Frank et al. 2005), and functionally, *reward contamination* overlaps the same region

(based on data in Wagner et al. 2007) and DA has been linked to processing reward and punishment (e.g., Matsumoto and Hikosaka 2009), these findings converge to support the plausible role of DA linked to reward contamination in AN (Keating 2009). Moreover, in the context that upregulated HPA-axis activity leads to stimulation and secretion of DA, it is likely that reward contamination (linked to DA) is stimulated and reinforced, and therefore maintained by upregulated HPA-axis activity in the illness.

Although estrogen has been largely dismissed as a diagnostic or clinical indicator for AN (e.g., Roberto et al. 2008), at puberty estrogen is proposed to contribute to sensitivity to stress-linked reward at the onset of AN. Paradoxically, however, evidence supports the hypothesis that estrogen also contributes to treatment response (Keating et al. 2010) consistent with its interactions with serotonin, the HPA-axis, and DA, postulated to facilitate response to medication that downregulates the HPA-axis.

2 Sex-Specific Psychobiological and Pharmacological Explanation for the Disproportionate Development of Anorexia Nervosa in Females: Stress and Reward Sensitivity Triggered by Estrogen at Puberty

2.1 Evolutionary Conservation: Females Are More Sensitive to Stress and Reward than Males

A complex interaction of factors likely contributes to a biological rationale for the diagnostic predominance of AN (up to 95% of cases, DSM-IV-TR 2000) among females. The disproportionate precipitation of the illness is postulated to reflect distinct evolutionarily conserved differences in females relative to males in *sensitivity to stress* and *reward* psychobiology, given stress is involved in the onset and maintenance of AN (e.g., Lo Sauro et al. 2008) and the majority of patients with AN demonstrate HPA-axis hyperactivity (Bergh and Sodersten 1996; Keating 2009; Licinio et al. 1996, among others) linked to illness behaviors (Bergh and Sodersten 1996; Keating 2009).

Physiologically, stress-induced upregulation of the HPA-axis produces elevated cortisol (reviewed in Tilbrook and Clarke 2006), the consequences of which include increased synthesis and secretion of reward-linked DA in AN (e.g., Bergh and Sodersten 1996). Females in general (clinically and preclinically) respond with greater perturbations of the HPA-axis than males in the context of psychosocial stressors (for review, see Tilbrook and Clarke 2006) and show greater sensitivity to reward than do males (e.g., Kamarajan et al. 2008) of which the *sensitivity to* the experience of reward is likely to be physiologically mediated via estrogen (e.g., Zakharova et al. 2009).

Although AN behaviors have been linked to stress-induced reward (e.g., Bergh and Sodersten 1996), the disproportionate diagnosis of AN in females linked to stress, reward and estrogen has until now not been formally hypothesized (neither in other stress-linked illnesses favoring females, e.g., major depression). Indirect support for the relationship between these physiological variables validates the potential for a relationship between these factors contributing to AN (Monteleone et al. 2001). For example, increased cortisol concentrations, but reduced concentrations of estrogens (i.e., 17β-estradiol) (among other hormones measured including dehydroepiandrosterone, DHEA), have been reported in AN patients (Monteleone et al. 2001), which is logically consistent with the impact of hyperactive HPA-axis-induced suppression of the HPG-axis.

Regarding the molecular dissemination of sex differences in stress and reward potentially contributing to AN, data from at least one psychobiological study can be extrapolated to address this relationship. Recently, differences in the stress, CRH system response to psychosocial stressor challenge were investigated between male and female sheep (Rivalland et al. 2007), and serendipitously for the purposes of the current contention, the positive control was enkephalin-staining, a reward-linked opioid. Following psychosocial isolation restraint stress, among results, female sheep demonstrated a greater proportion of enkephalin (reward-linked) cells staining for Fos than males in both control and stressed animals, suggesting (of particular relevance) *a greater volume of reward-linked cells in females*, which was present *before* the stress-induced challenge (i.e., independent of the effects of stressor). Extrapolated to the clinical condition, females may present with a greater proportion of reward-linked neural substrates opposed to the stress system (including those diagnosed with AN) predisposing females to the disproportionate expression of reward and stress-linked physiology and pathophysiology. Data from Rivalland et al. (2007) may also infer (although speculative) that females (relative to males) may experience dose-dependent greater reward from stress, on the basis of greater enkephalin Fos staining in females. Owing to the greater volume of reward-linked cells found in females in this study, it is plausible (at least indirectly) that estrogen may be putatively involved in mediating the difference in reward between males and females.

2.2 *Estrogen Mediates Increased Sensitivity to Reward in Females*

Evidence suggests that the mechanism mediating greater sensitivity in reward and stress systems in females is estrogen. Evidence from preclinical and clinical research (yet to be tested in patients with AN) suggests a strong rationale for endogenous estrogen concentrations contributing to the sex difference in the experience of reward favoring females (Zakharova et al. 2009; Fattore et al. 2007). In a recent study, younger female mice demonstrated greater behavioral *sensitivity* to rewarding stimuli (cocaine induced place preference) than older females or male littermates of any age (Zakharova et al. 2009), and consistent with estrogen's role in

mediating sensitivity to reward favoring females, when animals were ovariectomized, the sex difference in reward sensitivity was lost. Further support for estrogen involvement in reward sensitivity can be illustrated by a study demonstrating that certain female rat strains (both Long–Evans and Lister Hooded, but not Sprague–Dawley strains) experience greater reward than males, evidenced via greater self-administration of the cannabinoid (CB1 receptor) agonist (WIN 55, 212–2), in addition to which ovary-intact females experienced greater reward than estrogen-treated ovariectomized females (Fattore et al. 2007).

These preclinical findings are consistent with clinical research, demonstrating (though less directly) reward sensitivity favoring females. For example, in response to wins and losses on a gambling task, healthy females showed greater sensitivity to reward and loss (wins and losses, or punishment) than males (Kamarajan et al. 2008) in addition to which males were *equally sensitive* to both wins and losses. The neural correlates of these experiences were determined via brain oscillation, revealing that for females, greater reward sensitivity was linked to greater theta power and posterior maxima in the posterior region of the brain and activity in the anterior region when compared to loss conditions, linked to anterior involvement only. Males, however, demonstrated posterior maxima to both stimuli linked to activation in the frontal midline (Kamarajan et al. 2008). These results support an indirect role for estrogen in reward sensitivity, given females relative to males were more sensitive on relevant conditions of the gambling task. Future studies involving fMRI and PET imaging during ligand-specific binding (e.g., targeting DA) would resolve more specifically brain regions and circuits involved in the sex-specific experience of reward and punishment (or wins and losses), providing relevant insight regarding mechanisms that may lead to reward dysfunction (e.g., contamination) and neural substrates driving and maintaining behaviors linked to AN.

Although the impact of increasing levels of estrogen, characteristic of changes at puberty on the reward system, have yet to be investigated in AN, evidence for a modulatory impact of estrogen on reward systems can be extrapolated from a study disseminating the impact of concentrations of estrogens (e.g., estradiol) across the menstrual cycle in primates on the expression of the DA (reward-linked) system (Czoty et al. 2009). Specifically, during the luteal phase (high estradiol and progesterone concentrations) there was greater availability of D2 binding sites (via [18] FCP binding) in the caudate and putamen (e.g., striatum), suggesting greater unoccupied receptors in primates during physiological periods of higher estradiol (and progesterone) (Czoty et al. 2009) which has been suggested to reflect *reduced DA* release during the high estradiol phase (Young and Becker 2009).

Extrapolated to AN, it would be logically consistent that when estrogen levels are chronically low, DA levels are likely high. This can be illustrated by evidence that DA levels in the illness are elevated, as assessed via eye-blink rate (e.g., Barbato et al. 2006), a peripheral marker of central DA activity in patients relative to healthy controls. Furthermore, empirically, in weight-restored patients relative to healthy controls, D2 and D3 receptor binding has been shown to be increased (via [11C] raclopride binding) in the anteroventral striatum (Frank et al. 2005). Although menstrual cycling was not controlled for, it might be that increased D2

and D3 receptor binding reflects at least in part an impact of *estrogen normalization* on D2 binding [given three normal cycles were required for inclusion in the study by Frank et al. (2005) (estrogen normalization and treatment response discussed in Keating, et al. 2010)]. These interpretations are logically consistent with the physiological reports from Czoty et al. (2009) demonstrating that higher levels of estrogen are associated with lower concentrations of DA (Young and Becker 2009) and greater D2 and D3 receptor availability (Czoty et al. 2009).

2.3 *Females Are More Vulnerable to Anorexia Nervosa Due to Estrogen-Mediated Stress and Reward Sensitivity*

Support for sex differences in *susceptibility to development of AN behaviors* favoring females (Hancock and Grant 2009) linked to *stress and reward* (e.g., Bergh and Sodersten 1996) has been demonstrated in an activity-based rodent model of anorexia nervosa (ABA). Specifically, females were significantly more susceptible to restricted feeding and excessive wheel running, as demonstrated by more rapid and pronounced reductions in body weight and lower levels of food intake, when compared to males (Hancock and Grant 2009). Although endocrine and neurochemical measures were not taken, sex differences in the effects of food restriction and wheel running in ABA likely reflect differences in stress (among other endocrinological) factors, consistent with findings that HPA-axis reactivity, i.e., levels of adrenocorticotropin hormone (ACTH)/corticosterone (the rat homologue of cortisol) are greater in females relative to males, at rest and following exposure to a range of stressors, including forced swim (Panagiotaropoulos et al. 2004; Wigger and Neumann 1999), motorized wheel running, immobilization, footshock (Kant et al. 1983), and the chronic mild stress paradigm (Dalla et al. 2005, see chapter x).

Taken together, these outcomes (e.g., Hancock and Grant 2009; Panagiotaropoulos et al. 2004; Wigger and Neumann 1999) are consistent with the majority of literature concerning greater responsiveness of the HPA-axis in females than males (Tilbrook and Clarke 2006), and heightened HPA-axis reactivity may provide a risk factor for greater impact of the *stress* (Hancock and Grant 2009) of a wheel-running stressor (or clinically relevant excessive exercising), and its reward- (DA) linked reinforcement.

To test whether estrogen is crucial in mediating the greater vulnerability of females to the development of AN, and the contribution of greater sensitivity to stress and reward-linked systems in this context, a similar paradigm presented in Hancock and Grant (2009) may be tested. In addition to the utilization of continually assessed HPA-axis and DA levels in males and females compared on vulnerability to the ABA model, a study could involve females that are ovary intact, or ovariectomized or ovariectomized with estrogen replacement, at *puberty*, to determine the contribution of estrogen to the greater vulnerability of ABA in females.

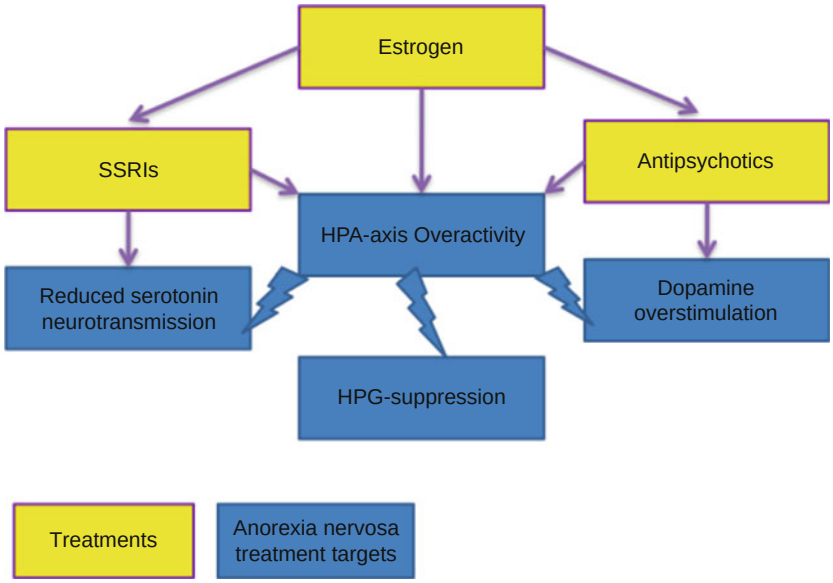

Fig. 1 A model for treatment targets in anorexia nervosa

2.3.1 Summary

In summary, in a model of ABA, females demonstrated greater sensitivity or vulnerability to the development of behaviors mimicking the AN clinical condition (excessive exercise and food restriction) that have been linked to elevated activity of the HPA-axis in clinical participants (e.g., Bergh and Sodersten 1996) and reflect elevated DA secondary to elevated HPA-axis activity in an animal model (Bergh and Sodersten 1996). These assertions are furthermore consistent with reports for the greater impact of *varied stressor tasks* on females relative to males described. Evidence supports the hypothesis that estrogen mechanistically contributes to the greater sensitivity to reward seen in females relative to males (e.g., Fattore et al. 2007; Zakharova et al. 2009), which may contribute to greater sensitivity around the time of puberty, in the development of stress-induced reward linked to behaviors, disproportionately precipitating AN in females.

3 Neuropsychopharmacology of Treatment Response in Anorexia Nervosa

3.1 The Estrogen Paradox

While estrogen has been linked to greater reward sensitivity in females relative to males, empirically rationalized to contribute to the disproportionate diagnosis of

AN in women, paradoxically, estrogen may facilitate response to pharmacological interventions including selective serotonin reuptake inhibitors (SSRIs) as well as antipsychotics via estrogen's contribution to HPA-axis attenuation, indirect influence on DA and contribution to serotonin modulation. The importance of estrogen in a clinical response to pharmacotherapy has been previously discussed (Keating et al. 2010). A schematic depicting neurobiological treatment targets for AN based on illness linked suppression of the HPG-axis (e.g., reduced estrogen levels), hyperactivity of the HPA-axis, elevated DA levels, and reduced serotonin levels is presented in Fig. 1.

Central to illness maintenance is HPA-axis overactivity. HPA-axis overactivity stimulates DA secretion, reinforcing (contaminated) reward-linked behaviors in AN. HPA-axis overactivity leads to a reduction in serotonin, the consequences of which facilitate HPA-axis overactivity. HPA-axis overactivity ultimately suppresses the HPG-axis, leading to amenorrhea. Normalizing this context, estrogen treatment can inhibit the HPA-axis (Young et al. 2001) estrogen is involved in the regulation of serotonin (Gundlah et al. 2005) and may facilitate the therapeutic effects of serotonin modulators (e.g., SSRIs) (Keating et al. 2010), ultimately facilitating attenuation of the HPA-axis, the consequence of which may normalize reward via reduced DA stimulation. Estrogen may also facilitate the actions of antipsychotics (e.g., antagonizing DA), reducing (contaminated) reward-linked reinforcement of disorder behaviors. According to this model, treatment approaches involving estrogen and SSRIs or estrogen and antipsychotics may reduce HPA-axis hyperactivity-induced reward linked to the reinforcement of AN behaviors.

3.1.1 Treatments in Anorexia Nervosa

The first line pharmacological approach to treating AN has involved the use of SSRIs which act to increase synaptic concentrations of 5-hydroxytryptamine (5-HT, serotonin). Albeit several small trials involving testing a large proportion of antidepressant treatments (75%) in patients with AN have demonstrated some improvement on measures including eating behavior and associated weight gain (and reduction in obsessive symptoms) (e.g., Rossi et al. 2007), there is an extensive body of literature demonstrating a lack of efficacy of antidepressant approaches in positively impacting desire for thinness or weight gain across a range of antidepressants including tricyclics, SSRIs, and mood stablizers (e.g., Attia et al. 1998; Biederman et al. 1985; Gross et al. 1981; Halmi et al. 1986; Kaye et al. 1998; Walsh et al. 2006). There are several explanations for their lack of efficacy, including the notion that abnormalities in the 5-HT system are not putatively linked to AN (e.g., Sodersten et al. 2008), and that (despite a lack of empirical support) enhancing 5-HT may lead to a dysphoric mood state (e.g., Kaye et al. 2009). Consistent with estrogen's regulation of 5-HT (e.g., Gundlah et al. 2005), a lack of estrogen in the illness state is proposed to mediate nonresponse to SSRIs (Keating et al. 2010).

3.2 *A Rationale for the Lack of Efficacy of Serotonin Modulators in Anorexia Nervosa: An Overlooked Mediator of Estrogen in Treatment Response*

The use of serotonergic modulators in AN is consistent with abnormalities in this system in patients, and dysfunction in this system has been extensively reviewed elsewhere (Barbarich-Marsteller 2007; Kaye et al. 2009; and others). For example, in recovered individuals with AN (restricting subtype), 5-HT transporter binding in the dorsoraphe nucleus (DRN) and anteroventral striatum is elevated (e.g., significantly increased [11C] McN562 binding potential assed via PET) relative to AN patients with bulimic characteristics (Bailer et al. unpublished, cited in Barbarich-Marsteller 2007). Reduced illness linked 5-hydroxyindolascetic acid (5-HTIAA) levels and elevated recovered state levels (reviewed in Barbarich-Marsteller 2007, logically consistent with elevated transporter binding in the illness state) would infer an association between recovery and augmentation of 5-HT levels; however, empirically medication enhancing 5-HT (e.g., SSRIs) show little benefit (Sodersten et al. 2008; Bergh et al. 1996; Adokat and Kutlesic 1995; Barbarich-Marsteller 2007) beyond facilitating relapse-prevention (e.g., Kaye et al. 2009). The neurobiological differences in recovered individuals relative to ill individuals, permitting some treatment efficacy of antidepressant medication, is likely linked to estrogen concentrations, but this has yet to be hypothesized or experimentally addressed.

The recovered state is a physiological status reflecting re-established estrogen concentrations, as opposed to the ammenorheic illness state. A difference in estrogen concentrations between these phases may explain the lack of efficacy of 5-HT modulators during the illness as opposed to recovery phase. Support for this contention can be derived from two lines of evidence: that 5-HT is regulated by estrogen (e.g., Gundlah et al. 2005) and that a therapeutic response to SSRIs demonstrated in other related illnesses suggests that treatment efficacy is linked to greater endogenous estrogen concentrations in females (e.g., Pae et al. 2009). For example, where amenorrhea is unlikely to be diagnosed (e.g., in depressed patients that are not severely underweight), females respond more favorably to SSRIs than males (Morishita and Kinoshita 2008), and in women taking hormone replacement therapy (HRT) during menopause, response to SSRIs is greater than in women not taking HRT (e.g., Zanardi et al. 2007).

These converging lines of evidence suggest that a lack of efficacy of SSRIs in AN is likely due to severely reduced estrogen in the illness state (Keating et al. 2010), which is consistent with and supported by the fact that in the recovered state, SSRIs are more effective in preventing relapse (e.g., Kaye et al. 2009) when menstrual function is restored. In addition to the observation that 5-HT is regulated by estrogen (Gundlah et al. 2005), antidepressants have been shown to attenuate activity of the HPA-axis (Schule 2006); hence, estrogen may also facilitate recovery in AN via its proposed effects in downregulating the glucocorticoid stress response (Solomon and Herman 2009), the consequence of which, in the context of AN, leads to reduced stimulation of DA.

3.2.1 Estrogen Reduces Activity of the HPA-Axis

Key to addressing the pathophysiology of AN is reducing or *hypoactivating* overactivity of the HPA-axis, the consequences of which may reduce DA synthesis and secretion, ultimately reducing reinforcement in AN (Keating 2010).

Recent evidence from a study involving stress-responsivity in female rats suggests definitively that estrogen (but not progesterone) is an important inhibitor of the HPA-axis (Young et al. 2001).

Consistent with HPA-axis inhibiting effects of estrogen (Young et al. 2001) and the corollary of reduced estrogen *activating* the HPA-axis, evidence shows that in females undergoing restraint stress, estrogen antagonists (tamoxifen and C1628) increase responsivity of the HPA-axis; that is, *C1628* increased both ACTH and corticosterone, and *tamoxifen* increased ACTH. Conversely, in ovariectomized female rats, low dose estradiol (over 7 days) *decreased HPA-axis responsiveness* (e.g., reduced ACTH) to psychosocial restraint stress, whereas progesterone had no effect (Young et al. 2001) (discussed elsewhere, Keating et al. 2010), which is consistent with the fact that both estradiol and progesterone reduced ACTH but that this magnitude did not differ when estradiol treatment was given alone (Young et al. 2001). These findings provide strong evidence for a modulatory impact of estrogen on stress-attenuation and warrant follow-up in AN.

3.2.2 Estrogen Regulates Serotonin and Facilitates Therapeutic Response to Serotonin Modulators

Females respond more favorably than males to SSRIs (Morishita and Kinoshita 2008), an effect likely mediated by estrogen. Data from primate and rodent investigations provide evidence that estrogen regulates 5-HT (e.g., Gundlah et al. 2005). For example, in females, 17β-estradiol (E2) likely induces tryptophan hydroxylase (TPH) (5-HT-precursor) expression in the DRN (the largest 5-HT containing nucleus in the brain) of guinea pigs and macaques (e.g., Bethea et al. 2000; Lu et al. 1999) and in murine DRN (Gundlah et al. 2005). In addition, estrogen regulation of TPH (subtype 1) may be specific to the estrogen receptor (ER) β-subtype, on the basis that estrogen increases TPH (subtype 1) expression (selectively) in the DRN of wild-type and ER-α knockout mice, where this effect on TPH (subtype 1) is not seen in ER β-knockouts (Gundlah et al. 2005).

At least in the context of major depression, which similarly to AN presents with significant abnormalities in 5-HT function and HPA-axis hyperactivity; endogenous concentrations of estrogen have been shown to influence response to 5-HT modulators. In a recent study, Zanardi et al. (2007) have demonstrated that menopausal women taking HRT (despite complex drug combinations between patients) reported a significant improvement in response to treatment (and reduction in depressive symptoms). These findings are consistent with other reports for greater endogenous concentrations of estrogen favoring response to 5-HT modulators (e.g., Pae et al. 2009; Morishita and Kinoshita 2008), consistent with the finding that

females respond to antidepressants more favorably than males (Morishita and Kinoshita 2008 and see chapter by Sramek and Cutler).

Although yet to be experimentally addressed in patients with AN, a lack of empirical support for the efficacy of medication that enhances 5-HT (e.g., SSRIs) (Adokat and Kutlesic 1995; Barbarich-Marsteller 2007; Bergh et al. 1996; Sodersten et al. 2008) beyond facilitating relapse-prevention (e.g., Kaye et al. 2009) in AN may be due to a lack of estrogen. In sum, estrogen may facilitate SSRI (i.e., 5-HT) induced hypoactivation of the HPA-axis, the consequences of which reduce DA stimulation and reward-linked behavioral reinforcement.

3.3 A Rationale for the Greater Clinical Response to Antipsychotics Versus SSRIs in Anorexia Nervosa?

Antipsychotic medication has more recently begun being trialed in patients with AN, and evidence suggests that antipsychotics show promise in the treatment of AN albeit further treatment controlled trials are necessary to conclusively support their widespread clinical use (Court et al. 2008).

In the context of the current neurobiological explanation for AN, it is plausible that antipsychotics show enhanced efficacy over SSRIs on the basis that they antagonize DA receptors, which may theoretically reduce the reward-linked reinforcement associated with illness behaviors. DA blocking is a direct target of antipsychotics, whereas it is a downstream consequence of SSRIs, that is, achieved via a consequence of HPA-axis downregulation. Assuming reward-linked reinforcement is key to illness maintenance (via maintaining reward-conflict) in some patients, an approach directly targeting this system may be warranted, whereas in patients with lesser reward sensitivity (e.g., less excessive exercisers) SSRIs and estrogen may be appropriate (Keating et al. 2010).

The therapeutic value of antipsychotics in AN may furthermore be augmented by an estrogen adjunctive. This assertion is supported by evidence demonstrating, *physiologically*, a link between normalization of estrogen levels in recovered AN patients and DA, and is consistent with primate literature demonstrating that greater concentrations of estrogen (according to the luteal phase of the menstrual cycle) in association with greater availability of D2 binding sites (or unoccupied receptors during *relatively* elevated estradiol) (Czoty et al. 2009) and reduced DA release [consistent with findings from (Czoty et al. 2009) discussed in (Young and Becker 2009)]. So while in healthy primates, higher levels of estrogen are associated with lower concentrations of DA (Young and Becker 2009) based on the findings by Czoty et al. (2009) in AN, the opposite is seen. That is, concentrations of DA are high (Barbato et al. 2006) (e.g., overactivity of reward) and estrogen levels are low (Monteleone et al. 2001) reflecting amenorrhea.

It will be important in future to establish the relative efficacy of SSRIs and estrogen versus antipsychotics and estrogen in this patient group.

4 Conclusions and Future Directions: Estrogen Augmentation of SSRIs or Antipsychotics to Induce/Facilitate Response to Treatment via Normalizing Stress Hyperactivity and the Experience of Reward

Given HPA-axis activity is significantly elevated in AN (e.g., Bergh and Sodersten 1996; Licinio et al. 1996) consistent with elevated DA concentrations (Barbato et al. 2006) and estrogen has been shown to inhibit the HPA-axis during psychosocial stress (Young et al. 2001), as well as regulate 5-HT metabolism (Gundlah et al. 2005), it is logically consistent then that an AN-linked deficit in estrogen concentrations may contribute to elevated activity of the HPA-axis, and furthermore enable hyperactivity due to disadvantaging the capacity of 5-HT to attenuate the HPA-axis. Taken together, reduced estrogen may explain a lack of response to 5-HT modulation. The corollary of this explanation is that estrogen treatment in combination with SSRIs should be trailed in AN, in addition to which studies are required to assess the clinical utility of estrogen and antipsychotics in AN. These treatment directions are consistent with the theoretical proposal that stress-induced reward (albeit ultimately *contaminated* reward) underpins behaviors linked to the illness, and that estrogen-induced normalization of the HPA-axis may lead to the reduced reward-linked reinforcement of behaviors, in conjunction with SSRIs or antipsychotics.

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Gender Differences in Neurodevelopmental Disorders: Autism and Fragile X Syndrome

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Contents

Abstract Gender is an important factor to consider in understanding the clinical presentation, management, and developmental trajectory of children with neuropsychiatric disorders. While much is known about the clinical and neurobehavioural profiles of boys with neuropsychiatric disorders, surprisingly little is known about girls. The aim of this chapter was to review our understanding of gender by considering the most prevalent childhood onset neuropsychiatric disorders, autism and Fragile X syndrome. This chapter highlights findings which suggest that girls with autism and Fragile X syndrome show some unique differences in cognitive and clinical profiles when compared to boys with these conditions; this may indicate the need for innovative assessment and management approaches which take gender into consideration. Our understanding of how differences emerge in boys and girls with neuropsychiatric disorders is unclear, future

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research needs to focus on the role of biological maturation rates, sex hormones, and psychosocial factors in order to progress this field.

Keywords Autism · Cognition · Fragile X syndrome (FXS) · Genetic Disorder · Gender · Intellectual Disability · Mental Health · Neurodevelopmental Disorder · Social Functioning

Sex differences in the prevalence of psychiatric disorders in children and adolescents have consistently been identified in epidemiological studies beginning with the landmark Isle of Wight study (Rutter 1989). Approximately twice as many prepubertal boys have psychopathology compared to girls, but the rates become more equivalent in adolescence. Changes in rates are also influenced by the type of disorder. Males are more vulnerable to suffer from attention-deficit hyperactivity disorder (ADHD) and autism in childhood, but as boys mature ADHD may become less prevalent and conduct disorder and substance abuse more prevalent (Rutter et al. 2003). A meta-analysis of 26 population studies found that the prevalence of depression doubles from childhood to approximately 5.6% in adolescence and is higher in females (5.9%) than in males (4.6%) (Costello et al. 2006). More young girls than boys are likely to suffer from depression, and following puberty this female preponderance for anxiety increases to around twice that for males (Lewinsohn et al. 1998). The causes of these gender differences are unclear, but an interaction between biological, psychological, and social factors are likely (Rutter et al. 2003; Nolen-Hoeksema et al. 1999). Lewinsohn et al. (1998) suggest that the female vulnerability to anxiety is based on genetic factors, but others claim that vulnerability to depression and emotional problems in females is strongly influenced by the effects of socialization, sensitivity to interpersonal relationships, the sense of mastery, self regulation, and response to stress (Nolen-Hoeksema et al. 1999; Kennan and Shaw 1997). Epidemiological studies of populations of twins and more recently the application of molecular genetics in longitudinal studies provide a method to describe gene–environment interactions contributing to psychopathology (Foley et al. 2004). A particularly useful way to investigate the "nature versus nurture" effects of gender on psychopathology is through studies of disorders that have a strong genetic component (henceforth referred to as "neurodevelopmental disorders"). Approximately, 3% of children will be born with a neurodevelopmental disorder equating to approximately 650,000 individuals in Australia alone. The impact of "nature" versus "nurture" is infinitely more complex in neurodevelopmental disorders where the interaction between prenatal hormones, specific susceptibility genes, and risk or protective genes on the X chromosome operates (Rutter 2005). The genetic mechanisms that result in a higher prevalence of males with child-onset neurodevelopmental disorders, including those for which genetic etiology, are still not determined, such as autism, as well as those disorders for which the genetic origins are known, such as fragile X syndrome (FXS), allow investigation of gender-related differences in prevalence, clinical manifestation, and cognitive and neuropsychiatric profiles. However,

across the neurodevelopmental disorder literature, little attention has been paid to the way in which downstream biological maturation rates, sex hormones, and psychosocial environments differentially impact on the developmental trajectory for male and females.

The focus of this chapter will be to overview the clinical, cognitive, and neurobiological literature on gender differences in the most prevalent childhood onset neuropsychiatric disorders, autism and FXS, the latter being the most common cause of hereditary intellectual disability in males, resulting from the silencing of a single gene on the X chromosome. Thompson et al. (2003) have extensively reviewed the literature on gender differences in neurodevelopmental disorders and concluded that gender has yet to matter sufficiently in neurodevelopmental disorder research, and by extension clinical practice. The almost exclusive focus on males with neurodevelopmental disorders in the biological and clinical research literature has led to an imbalance in gender-specific information, which can inform the clinical assessment and management of females with neurodevelopmental disorders. In this chapter, we will highlight the important gender differences that necessitate careful investigation of male and female profiles across development to facilitate targeted gender-specific clinical and educational interventions and treatments.

1 Autism

1.1 Diagnosis

Autism is a generic term referring to a group of related conditions defined in the DSM-IV-TR (American Psychiatric Association 2000) and the ICD-10 (World Health Organisation 1992) as pervasive developmental disorders (PDD). These disorders have their onset within the first 3 years of life, but the clinical picture may change with development. In DSM-IV-TR (American Psychiatric Association 2000), the PDDs comprise the categories of autistic disorder, Asperger disorder, Rett's disorder, childhood disintegrative disorder, and PDD – not otherwise specified (PDD-NOS). Autism was first described by Leo Kanner in 1943 in a group of 11 children who had the distinctive core features of social, language, and communication disturbance, and an obsessive desire for sameness (Kanner 1943). In the following year, Hans Asperger described a group of 16 children and adolescents in Vienna who had deficits in communication and social skills together with obsessional interest, intolerance of change, and motor clumsiness (Asperger 1944). Unlike the children described by Kanner, these young people were of normal intellectual ability and did not have any delay or abnormality in their language development. This has become the differentiating feature of Asperger disorder from autistic disorder.

All children with autism have impaired social interactions, which may change as they develop. Infants with autism do not anticipate social interactions, such as being

picked up, or seek physical comfort or parental attention. Preschool children with autism usually avoid eye contact and do not engage in social imitation such as waving goodbye. They are unresponsive to the feelings and emotions of others. They are aloof and unable to engage effectively with other children or understand reciprocal social interactions. As the child grows older, there may be an increased interest in other people, but social skills are often stilted and learned in an inflexible manner, leading the child to appear odd and socially clumsy. Parents usually first seek help because their child has language delay and a lack of nonverbal communication, and easily becomes frustrated. About 50% of children with autism fail to develop functional speech and only learn slowly to compensate with gesture. Language development is often abnormal in the remainder, with echolalia, self-directed jargon, and the repetition of irrelevant phrases, for example from a TV show. The correct use of pronouns and the related development of a sense of self and others are delayed. Poor comprehension, problems expressing needs by words and gesture, and difficulty in social understanding are frequently the causes of frustration and disturbed behavior. Children who do develop functional language usually have difficulty in using language socially and in initiating or sustaining a reciprocal conversation. For example, the child may talk at others in a socially inappropriate manner. In contrast to children with autistic disorder, young people with Asperger disorder have no delay in the development of normal expressive and receptive language, including the use of communicative phrases by the age of three. However, children with Asperger disorder have problems in their social use of language, for example being verbose and preoccupied with a favorite topic. Their speech may appear odd due to the use of an unusual accent, or the presence of abnormalities in pitch and volume, leading, for example, to a flat and monotonous delivery.

The play, behavior, and daily life of children with autism are usually rigid and repetitive. Younger children may line up toys or objects, or be preoccupied with special objects such as stones, and become distressed if these activities are interrupted. Their ritualistic play lacks imagination and social imitation. With development, play may become more complex, such as re-enacting scenes from a favorite video story, but is usually still repetitive. The older child may develop preoccupations with themes such as train timetables or dinosaurs, and this will be the focus of their play, drawing, and conversation. They may have a number of rituals associated with daily life, such as a fixed order for bathing and dressing, or an insistence on wearing the same clothes or taking the same route to a familiar place. Change or unexpected events can be distressing: for example, the arrival of a new student in the classroom. There may be a number of perceptual or sensory abnormalities such as hyperacusis or tactile sensitivity, manifesting, for example, as an aversion to having their hair brushed. Some children with autism have a remarkable lack of sensitivity to pain. Children with autism are usually visually attentive: for example, they may study the detail in a picture book or closely observe spinning wheels, the edges of objects, or reflections in water. There are usually some motor mannerisms, such as hand flapping, or tiptoe walking and gait abnormality.

Approximately 80% of children with autism also have intellectual disability, and a range of other emotional and behavioral disturbances is common. Children with autistic disorder who have intellectual abilities within the normal range are referred to as high functioning. The individual cognitive profiles of children with autism usually show a wide scatter of abilities, with deficits in verbal and social comprehension tasks, and more ability with visuomotor performance skills. In contrast, children with Asperger disorder have overall normal intellectual abilities, but usually have relative deficits in visuomotor tasks and motor skills compared with their verbal performance. For the sake of brevity, autism and Asperger disorder will henceforth be referred to as "autism."

2 Fragile X Syndrome

FXS was first described in 1943 by Martin and Bell (originally labeled the "Martin-Bell" syndrome) and is the world's most common hereditary cause of developmental delay in males. The disorder is caused by the silencing of a single gene on the long arm of the X chromosome at q27.3. The gene, named the Fragile X Mental Retardation Gene-1 (FMR1), was identified in 1991 and is "turned off" in affected individuals. When this occurs, there is an expansion of a trinucleotide (CGG) in the repeat region. In individuals unaffected by FXS, there are between 7 and 55 CGG repeats, with 30 repeats the most common number. In clinically affected individuals (known as the full-mutation status), the CGG region expands to over 200 repeats, resulting in silencing of the gene and loss of the fragile mental retardation protein (FMRP). The extent to which these molecular discoveries explain the behavioral phenotypic outcomes in FXS is beginning to be revealed (see also Cornish et al. 2008, for a recent review of the genetic-cognitive/behavioral correlates that comprise the fragile X continuum).

The clinical features that can characterize FXS include an elongated face, large prominent ears, and forehead, and in males, postpubertal macroorchidism (Cornish et al. 2007a; Lachiewicz et al. 2000). More subtle features can include narrow inter-eye distance, a highly arched palate of the mouth, and hyperextensible joints. However, the wide variability in manifestation in both boys and girls makes a diagnosis based on physical features alone almost impossible. It is precisely because of their relatively "normal" appearance that many affected children are not diagnosed with FXS until relatively late in their development. Undoubtedly, the most defining feature, especially in boys with the disorder, is intellectual disability and the resulting cognitive-behavioral phenotype, most notably the attentional control difficulties, language, and spatial impairments that can accompany the syndrome from very early in development (Abbeduto et al. 2007; Cornish and Wilding 2010; Cornish et al. 2007b; Scerif et al. 2004, 2007).

Undoubtedly, behavioral problems often link with cognitive impairment, and two disorders in particular that co-exist in many individuals with FXS are autism and ADHD. Due to the pervasiveness of these symptoms, a diagnosis of FXS may

occur later in childhood or never at all if clinicians are unable to dissociate the FXS phenotype from a diagnosis of autism or ADHD. To address this concern and provide much needed information to clinicians and educators, the findings from a series of recent studies have begun to elucidate the FXS "signature" associated with both autism and ADHD.

Among the most distinctive and pervasive behavioral features of young boys with FXS are attentional and hyperactivity problems (Cornish et al. 2001a; Hatton et al. 2002; Sullivan et al. 2007), the severity of which often leads to a clinical diagnosis of ADHD. At a finer-tuned level, however, the FXS profile appears to be characterized by unexpectedly extreme levels of inattentiveness, restlessness, fidgetiness, impulsive tendencies, and distractibility even when their level of general development is taken into account. In one of the most comprehensive study's to date, Turk (1998) compared the ADHD profiles of 49 FXS boys (aged 4–16 years) to that of 45 boys with Down syndrome (aged 4–16 years), and 42 boys with mental retardation of an unknown cause (aged 4–16 years). Although both groups of boys showed similar levels of motor activity, the boys with FXS show significantly more inattentiveness, restlessness, fidgetiness, distractibility, and impulsive tendencies suggestive of DSM-IV ADHD predominantly inattentive type. Moreover, there is some evidence that these features do not necessarily improve with age (in contrast to most children with these traits), emphasizing the need for early diagnosis and multidisciplinary intervention. In girls with FXS, the research is not as extensive as that undertaken in affected boys but nonetheless points to a substantive minority of girls presenting with ADHD-inattentive type symptoms (Mazzocco et al. 1998). Together, these findings highlight a distinctive ADHD profile in the FXS full mutation that is not solely the artifact of intellectual disability.

One of the most intriguing and complex of relationships in FXS is its association with autism. There are currently very few single-gene disorders for which there is a certainty of the involvement of autism; FXS is one. As a single-gene disorder, FXS offers an interesting genetic model to explore the functions of FMRP regulation and the repercussions of its loss in early brain development. Commonalities across core social and language domains define the link between FXS and autism, and it therefore seems highly plausible that similar neurobiological mechanisms are affected in both disorders. A recent study by Loesch and colleagues found that a common impairment in verbal skills best described the comorbidity of FXS and autism at the cognitive level (Loesch et al. 2007). In an earlier study, Philofsky et al. (2004) reported a similar link in children with FXS between exceptionally low verbal ability, in this case receptive language, and a dual diagnosis of autism, compared to children with FXS alone in which verbal skills appeared to be a relative strength. Overall, children with a dual diagnosis tend to display more impaired cognitive performance than children with either autism alone or FXS alone.

However, although commonalities between FXS and autism appear quite striking at one level, subtle differences are notable. For example, in terms of eye gaze, in children with autism atypical eye gaze is most acute in social interactions

and appears to be motivated both by a lack of understanding of the social situation itself and by the absence of a desire to communicate. In contrast, eye gaze behavior in FXS does not appear to be guided by a lack of social awareness or communication. The majority of individuals with FXS, although tending to avoid social interactions, will offer what is now classically termed the "fragile X handshake," whereby an initial wish to communicate socially, with a "handshake," a socially acceptable remark or even brief initial eye contact, is coupled with active and even persistent gaze avoidance. Subsequent interactions with familiar persons may be marked by the same active gaze avoidance despite the growing relationship. The gaze avoidance persists even when attempts are made to extinguish it; it may, in fact, increase in intensity. It has been suggested that FXS is associated with a unique pattern of hyperarousal and social anxiety that can cause them to avert their eyes in a social situation (to avoid the sensory stimulation of eye contact) but may still wish to communicate socially (Cornish et al. 2004a; Wolff et al. 1989). Thus, individuals with FXS are more likely to exhibit autistic-like behaviors such as eye gaze aversion, which are more symptomatic of their hyperarousal and social anxiety rather than from an inherent lack of understanding of the social situation. See Cornish et al. (2007a) for more detailed descriptions of the commonalities and differences between FXS and autism across cognitive domains.

The frequency of autism among FXS individuals is still controversial, but approximately 2–6% of children with autism will have the FMR1 mutation (Reddy 2005; Wassink et al. 2001), and between 33% and 67% of children with FXS will fulfill the diagnostic criteria for autism (Clifford et al. 2007; Rogers et al. 2001) with more males than females reportedly meeting the autism cut-off.

3 Autism: Prevalence and Gender Differences

It is well established that there is a greater preponderance of males than females with autism. The magnitude of gender ratio differs with intellectual ability. For children with autism who have associated intellectual disability (referred to in the literature as low-functioning autism, LFA), the gender ratio is approximately 2:1. For children with autism who have above-average intellectual abilities (referred to as high-functioning autism or Asperger disorder), the ratio goes up to 10:1 (Baron-Cohen et al. 2005).

There is evidence that the prevalence of autism is increasing. Fombonne, DuMazaubrunc, and Grandjean (Fombonne 2003) reported rates of 10–12 per 10,000 using rigorous diagnostic criteria and standardized diagnostic assessment. Prevalence estimates increase substantially when the broader autism spectrum is included, and a broader range of assessment techniques are used. In Australia, the estimate of around 1 in 160 was given for children between the ages of 6 and 12 years (Australian Advisory Board on Autism Spectrum disorders). Similar figures have been reported by the US Centre for Disease Control (CDC 2007), estimating a prevalence figure of 1 in 150, for 8-year-old children diagnosed with an "autism

spectrum disorder." Liu et al. (2010) recently reported prevalence clusters of children with autism which related to groups of parents who were more informed about the condition through knowing a family or affected child with autism within the community. Liu et al. (2010) noted that these parents were more likely to seek a formal diagnosis, than those parents who had not had community contact with the diagnosis of autism.

4 Fragile X Prevalence and Gender Differences

FXS affects approximately 1 in 2,500 males (Hagerman 2008). The focus initially in females with FXS has been on those with significant cognitive deficits. However, the spectrum of involvement includes those with learning difficulties and/or emotional problems with an IQ in the broad range of normal. Since most females with the full mutation fall in this category, the prevalence of affected females including this milder involvement will be greater than 1 in 8,000. In the FXS full mutation (>200 repeats), X linkage means that males are especially vulnerable to the full effects of the condition at the brain, behavioral, and cognitive levels. Almost all boys will present with moderate intellectual disability with profiles emerging as young as 3 years of age (Skinner et al. 2005). In females, the phenotypic variation is such that some girls only show subclinical learning disabilities (Bennetto and Pennington 2002), while approximately 25% display more significant cognitive impairment (most with mild ID and rare individuals with moderate ID) similar in profile to boys with FXS. Genetic variation in the form of X-inactivation (when one of the two X chromosomes remains inactive and the other active) is seen as the major contributor to the heterogeneity of intellectual disability and the broad range of cognitive deficits seen in females with FXS. For obvious reasons, this is not an issue of concern in FXS males whose impairment, without the protection of X-inactivation, shows greater severity. In males, FMRP level accounts for about 75% of the variance in IQ, but in females the proportion is much smaller (Lightbody et al. 2006).

5 Autism: Clinical Presentation and Gender Differences

There is no evidence that the core symptoms of autism differ for males and females. Volkmar et al. (1993) compared males and females with autism on the Autism Behavior Checklist, age of onset, and ratings of ICD-10 (World Health Organisation 1992) items. They found no consistent gender differences between males and females that were not related to intelligence (IQ). These findings were consistent with those of Pilowsky et al. (1998) who found no gender differences in a matched

sample of 18 males and 18 females on two commonly used autism assessment tools, the ADI-R (Le Couteur et al. 1989) and the Childhood Autism Rating Scale (Schopler et al. 1988).

While the core symptoms of autism are the same in males and females according to DSM-IV-TR criteria, the clinical picture may differ in a way which is consistent with gender-related differences in the normal population of male and female children. For example, differences in emotional expression, play interests, language, cognitive profile, and social relationships (Thompson et al. 2003) may give rise to differences in the way males and females with autism present clinically. While boys may have an intense preoccupation with vehicles, such as trains and cars, girls may develop their special interests in areas such as, teddy-bear or doll collections, and spend unusual amounts of time on craft, and art, to the exclusion of other types of play (Gillberg and Coleman 2000). Gender-neutral activities such as "drawing" may form the "special interest" for either males or females with autism. Gillberg and Coleman (2000) reported that females tend to have fewer special interests than males with autism, although this may relate to male interests being more circumscribed (e.g., trains, cars) than female interests (e.g., craft, doll play), and thus more clinically salient.

Normally intelligent females with autism may present as more passive, socially competent, and with better communication skills than boys. This may again link to what we know about gender differences in males and females in the typically developing population. For example, Skuse (2009) reported that among children who have higher verbal intellectual abilities in the general population, males are more vulnerable than females to deficits in social-communicative functioning. If we extend these typical gender differences to the autism population, we would expect that females would present more competently than IQ-matched males with autism who may be more obviously socially awkward. The explanation for females with autism presenting as more socially competent may be more simply explained in terms of gender differences in biological maturation rates which affords developmental advantages for females, but not males with autism.

While the clinical presentation of females with autism may appear superficially different to that of males, females experience the same, if not greater, levels of social isolation and disadvantage as males with autism. Using the Autism Diagnostic Interview (ADI) items, McLennan et al. (2005) reported that females were described as having more severe social deficits than males with autism. These social deficits were particularly evident during adolescence in peer relationships with none of the females in the study having any sort of reciprocal friendship after age 10. Holtmann et al. (2007) reported that females have more social, thought, and attentional problems than matched boys with autism as rated by their parents using the Child Behavior Checklist. However, Holtmann et al. (2007) suggest that these parent reports may themselves represent a bias in behavioral expectations for males and females, rather than a "true" gender difference in psycholopathology and behavioral functioning (Rutter 2005).

The superficially more competent social functioning of females with autism may result in a late diagnosis or misdiagnosis of the disorder. Gillberg and Coleman

(2000) reported that normally intelligent females with autism are referred to clinical services later than males with high-functioning autism. This finding may fit with the common referral pattern to psychiatric/psychological services to preference males who tend to have higher levels of disruptive behavioral disorders which impact more broadly on the family and school setting, than females who tend to have higher levels of internalizing symptoms. Children with autism who present with a passive and often "dreamy" clinical presentation, and also experience learning difficulties at school, may initially be given a diagnosis of attention-deficit disorder (ADD). It may be that this referral pathway is more common for females than males with autism, given the gender-mediated differences in clinical presentation, although there is no data to specifically make this distinction in diagnostic pathway. Other potential misdiagnoses for females with autism include a primary anxiety disorder, selective mutism, and anorexia nervosa (Thompson et al. 2003). Another potential clinical pathway is for females with autism to be initially assessed as having an insecure attachment disorder; this presentation together with the often overprotective parenting response it elicits (Soppitt 2006) may erroneously lead to psychological interventions focused on parenting or family functioning, rather than appropriate autism-focused intervention. The focus on "over" parenting to account for the insecure attachment presentation of girls with autism has many parallels with the "refrigerator," "under" parenting hypothesis used to account for the aloof and detached presentation of boys with autism in the 1950s.

6 Fragile X: Clinical Presentation and Gender Differences

Severity of clinical/behavioral difficulties in FXS males and females will vary across and within gender. For example, hypersensitivity and hyperarousal are recognized as early prominent behavioral features of children with FXS, with and without autism (Belser and Sudhalter 1995; Miller et al. 1999). For example, in girls with FXS, Keysor et al. (2002) demonstrated higher arousal levels at baseline relative to girls with Turner syndrome and typical developing control children, with a small rise on anxiety-provoking cognitive tasks compared to that seen in girls with Turner syndrome, resulting in eventual comparable anxiety in both groups on these tasks. In terms of ADHD symptomology, there is a well-documented profile in males (Asperger 1944), but in contrast in FXS girls, the profile is less well documented with more variability. Unlike affected boys, only about one-third of girls appear to meet the DSM-IV diagnostic criteria for ADHD although many will present with some ADHD symptomology, notably inattentiveness rather than hyperactivity (Mazzocco et al. 1998). For recent reviews, see Cornish et al. (2004b) and Hatton et al. (2009). However, it is also likely that gender differences in clinical presentation will be related to severity of IQ and the presence or absence of comorbid autism.

7 Autism: Cognition and Gender Differences

In addition to environmental (e.g., parental expectation) and biological explanations (e.g., biological maturation rates) which may account for gender differences in autism, there may be a neuropsychological explanation for differences in clinical presentation between males and females.

Early studies comparing cognitive functioning in males and females with autism reported no noteworthy gender differences other than the level of intelligence (Volkmar et al. 1993; Tsai and Beisler 1983; Pilowsky et al. 1988). In one of the earliest studies of gender differences in autism, Lord et al. (1982) compared male and female children (3–8 years) on a range of nonverbal measures including intelligence, adaptive functioning, receptive vocabulary, perception, and eye–hand integration, as well as on affect, play, and relating human interest. While males were found to exhibit more unusual visual responses and inappropriate stereotypical play than females, the females in the study were found to be more impaired on every measure relating to cognitive functioning than boys including IQ, the Vineland social quotient, receptive vocabulary, eye–hand integration tasks, and perceptual skills.

More recent studies focused on normally intelligent children with autism (i.e., high-functioning autism or Asperger disorder) have indicated that gender differences may exist, laying suggestion for a gender-related neuropsychological phenotype (Kopp and Gillberg 1992). A recent study by Carter et al. (2007) comparing the cognitive profiles of toddlers with autism showed that males displayed stronger verbal and motor skills than girls, whereas girls exhibited better nonverbal problem-solving ability than boys. In the most recent study on gender differences in autism, Koyama et al. (2008) compared the cognitive profiles of children with high-functioning autism using a Japanese version of the Wechsler Intelligence Scale for Children – Third Edition. While no differences were observed in overall IQ scores, gender differences were observed on particular subtests: females outperformed males on tests of processing speed, coding, and symbol search, whereas boys were significantly better than girls on the block design subtest.

Nyden et al. (2000) have shown that females with autism have more impaired executive functioning when compared to males. Lemon et al. (2010) recently showed that females with autism have significantly more impaired executive functioning, in particular, response inhibition, when compared to typically developing females. No differences were found between males with and without autism on this test of executive function.

The subtle profile and intellectual differences between males and females are largely consistent with the only brain imaging study to look at gender differences in autism. Using magnetic resonance imaging, Bloss and Courchesne (2007) found that when males and females are matched on verbal and nonverbal intellectual ability, there was no difference in the pattern of brain abnormalities; however, females were associated with additional abnormalities in cerebral white and grey matter, and temporal grey matter (Bloss and Courchesne 2007). It was suggested

that these neurological gender differences may index etiological and downstream biological maturational differences in males and females with autism. Such neurobiological differences may also account for some of the suggested phenotypical gender-related differences between males and females with autism. More significant white and grey matter abnormalities may account for Gillberg and Steffenberg's (1987) earlier finding that being female puts an individual with autism at greater risk for behavioral and or cognitive deterioration postpuberty, with 12% of males and 50% of females showing deterioration in one or both domains. Minshew (1991) refers to puberty as marking a "second-wave" of frontal deficits for young people with autism: Future research should focus on different trajectories of fronto-striatal executive impairment for females and males with autism pre- and postpuberty. Put in a clinical context, mapping of executive dysfunction for females across this critical developmental period may provide an important clinical indicator of risk for postpuberty clinical deterioration. This type of research would help clarify whether clinical reports of high levels of frontally mediated psychopathology [e.g., social, thought, and attention problems (Holtmann et al. 2007)] are best accounted for by *"nature"* (e.g., parental expectations for females) or *"nurture"* [e.g., increased frontal-susceptibility in females; see Lemon et al. (2010)].

It will also be important for future research to feed-in what we know from the typically developing literature about differences in cognitive processes which characterize males and females. For example, higher levels of psychopathology in females with autism may relate to Canli and Amin's (2002) finding that females are better than males at remembering emotional issues and events; this possibly gender-mediated cognitive predisposition, accompanied with the obsessional thought patterns which are core to autism, may result in a tendency for females to ruminate more and become increasingly mentally unwell over time.

8 Fragile X: Cognition and Gender Differences

At the intellectual level, the majority of FXS males (>95%) will display IQs within the moderate-to-severe range of impairment, with profiles emerging as young as 3 years of age. In females, the phenotypic variation is such that some girls only show subclinical learning disabilities, while approximately 50% display more moderate-to-severe mental retardation similar in profile to boys with FXS. As already noted, the X-inactivation status (ratio of normal alleles on the X chromosome) in FXS females is seen as the major contributor to the heterogeneity of intellectual disability and cognitive deficits. Surprisingly, however, few studies have addressed the issue of whether degree of intellectual impairment changes with age, albeit progressing at a slower pace compared to typically developing children, or whether intellectual level actually decreases with increasing age, or even remains static across developmental time. Although published studies have consistently observed a decline in cognitive abilities from middle-late childhood onwards (Fisch et al. 1999, 2002), Cornish et al. (2004b) have previously argued

that this decline might possibly be due to the FXS child's increasing problems in maintaining and developing successful cognitive strategies that keep pace with their age-normed cohort rather than an actual regression in intellectual level or failure in neural development. However, few, if any, studies have examined intellectual decline and its relation to cognition in females, and fewer still have examined any profile beyond late childhood.

At the cognitive level, at least in males by late adulthood, relative strengths in vocabulary (Roberts et al. 2007), long-term memory for meaningful and learned information (Munir et al. 2000), and visual-perceptual skills (Cornish et al. 1999; Kogan et al. 2004) are accompanied by relative weaknesses in the storage and manipulation of complex information in working memory (Munir et al. 2000; Lanfranchi et al. 2008), linguistic processing (Abbeduto et al. 2007; Belser and Sudhalter 2001), visuo-spatial cognition (Cornish et al. 1998, 1999), and inhibition (Cornish et al. 1998, 1999). Disappointingly, there is an imbalance in the ratio of male to female studies with a greater proportion of research focused on the male phenotype as cited above. There is also a paucity of studies that have investigated cognitive *signatures across* both genders in the same research design.

In the domains of *attention* and *working memory,* toddlers and children with FXS when compared to typically developing children have significant impairments in attention and memory functioning. An early study by Hooper et al. (2000) showed that children with FXS from 4 years of age display striking difficulties in attention and memory subscales of the Leiter International Performance Scale-Revised, a nonverbal assessment tool. In a series of more recent studies, Scerif et al. (2004) have demonstrated even earlier difficulties in the control of attention in children with FXS as young as 12 months of age (Scerif et al. 2004, 2007). These studies aimed at tracing developmental trajectories of attentional control in both children with FXS and in typically developing groups. While typically developing toddlers displayed gradual improvements in the accuracy with which they searched their visual environment, toddlers with FXS tended to perseverate and were unable to shift attention away from previously correct responses, regardless of their overall developmental level. These findings replicate the pattern of difficulties seen in older boys (7–12 years) (Wilding et al. 2002) and in young adult men (18–30 years) (Cornish et al. 2001b). Similar difficulties in attention switching in FXS girls (8–16 years) have also been reported (Kovar 1993). Thus, difficulties in perseveration and in shifting attentional focus are core deficits in FXS and appear to remain constant with age. In contrast, the ability to select relevant from irrelevant information (selective attention) is a relative strength at least in FXS males that continues to develop linearly with increasing chronological age (Cornish et al. 2007b). Comparable developmental studies in females are needed to understand the range of attention dysfunction and its relation with age and IQ. To date, however, the current findings underscore the importance of recognizing and treating early attention deficits that if left untreated will impact significantly across development and learning in both males and females. It is of note that a pervasive deficit in attentional control may account for the prevalence of specific impairments across other cognitive domains that also involve inhibition as a core component, for example,

the high incidence of repetitive speech seen in boys and girls with FXS (Cornish et al. 2004b).

In the domain of memory, in boys there is evidence clearly pointing to *relative* strengths in long-term and short-term recall for meaningful information including memory for faces (Turk and Cornish 1998) and story recall (Munir et al. 2000) with performance at a level equivalent to typical children matched on *developmental* level (but not chronological age level). No equivalent published studies have been conducted in FXS girls. In terms of *working memory* (the ability to retain and manipulate information "online" over short periods which is crucial in guiding attention and behavior during the course of an activity), accumulating evidence points to a relative weakness in visuo-spatial working memory compared to verbal working memory (Munir et al. 2000; Cornish et al. 1999). For example, Munir et al. (2000) examined working memory performance in 25 boys with FXS aged 8–15 years, 25 boys with Down syndrome (trisomy 21) aged 7–15 years, and two groups (25 in each) of typically developing children matched to the syndrome groups on developmental level (mental age) and on chronological age. At first glance, the findings indicated general weaknesses across both verbal and visual memory skills that were not syndrome specific but suggestive of developmental delay. However, closer inspection revealed that the impairment of the FXS group relative to that of the DS group was significantly larger on tasks that tapped visuo-spatial memory skills than for tasks that tapped verbal memory skills. In comparison to FXS boys, few studies have addressed working memory in affected girls and those that have, focused almost exclusively on adult women. However, two recent studies by Mazzocco et al. (1998) and Kirk et al. (2005) highlight difficulties in working memory thresholds that also include a specific deficit in visual memory. Less than 53% of affected girls compared to 96% of typically developing females were able to recreate the gestalt of a design by memory even though they could correctly identify the object. This finding lends some support to a tentative hypothesis that visuo-spatial impairment may be a defining feature of the phenotype in both boys and girls irrespective of degree of intellectual impairment. However, one must show some caution here in giving the impression that visual memory is a global weakness in FXS. Variability especially in the female phenotype is inevitable, and studies of adult women have reported visual memory skills that are within the normal range (Mazzocco et al. 1993).

In one of the few FXS studies to date to assess both males and females on the same cognitive measures, Cornish and Wilding (2006) found evidence of gender-specific FXS "*signatures*" whereby males and females of similar verbal mental age display different cognitive profiles in the domains of spatial cognition and language. FXS males as a group performed worse overall, followed by FXS females and then typically developing controls. When gender differences emerged, they were only within the FXS groups. Females were superior to males on tasks that tapped visuo-spatial skill, visuo-constructive skill, and articulation, but both genders displayed comparable performance on tasks that involved visual perception such as gestalt integration and language comprehension. The pattern of *gender differences* in FXS represents a unique and important finding that highlights a

specific male weakness for skills that depend on visual and spatial construction of abstract, meaningless designs to form a whole, and for recall and construction of a familiar design. The fact that both tasks require relatively good visuomotor integration for successful completion may be the key to understanding the fragile X male deficit. Similar gender-driven studies across other core domains (e.g., attention and working memory) would be of tremendous benefit in adding to our knowledge about the impact of gender in X-linked genetic disorders.

9 Autism and Fragile X Gender Difference Theories

In FXS, genetic variation in the form of X-inactivation (when one of the two X chromosomes remains inactive and the other active) is seen as the major contributor to the broad range of cognitive deficits seen in females with FXS. This may also account for why in some females there is a significantly less impaired phenotype compared to their male counterparts who do not benefit from the protection of X-inactivation, and hence show greater severity.

Explanations for the gender differences in autism are less clear. Skuse (2000, 2009) and Baron-Cohen (Baron-Cohen 2002; Baron-Cohen et al. 2005) have proposed the two main theories to account for the preponderance of males versus females diagnosed with autism. Skuse (2009) proposed that males are more vulnerable to autism by virtue of a single X chromosome. Based on parallels between the high male preponderance of autism and social skill deficits in monosomy in females (XO), known as Turner syndrome, Skuse's theory suggests that a single X chromosome could reduce the threshold at which symptoms of autism manifest. Females with a second X chromosome have protection from the impact of autosomally mediated genetic vulnerability by "its influence on the development, structure, and function of the social brain" (Skuse 2009). Skuse (2009) proposed that X-imprinting would lead to male preponderance irrespective of gender hormones. This theory is proposed to account for the overrepresentation of males diagnosed with neuropsychiatric disorders, including ADHD, a disorder which like autism involves in social communication impairment (see a discussion of ADHD gender issues below) (Skuse 2000).

The evidence about whether X-linked genes contribute to individual risk factors for autism is controversial (Gong et al. 2008). One of the main limitations of Skuse's theory is that it cannot explain father-to-son transmission (Rutter 2005). Rutter (2005) noted that if an imprinted gene of the X chromosome is responsible for an increased male liability, it is likely that it operates in all males rather than through variable allelic transmission. Rutter (2005) suggests that "epigenetic effects remain a possibility and it is possible that prenatal hormones interact with susceptibility genes, but this possibility can be investigated more satisfactorily once susceptibility genes have been indentified" (Rutter 2008).

While Skuse's theory discounts a role for male sex hormones in the etiology of autism, Baron-Cohen's extreme male brain (EMB) theory (Baron-Cohen et al.

2005; Baron-Cohen 2002) proposes that exposure to higher levels of fetal testosterone (fT) may be an important causal factor in the etiology of autism, and could explain the male preponderance. The cognitive sequelae to increased testosterone in utero is hypothesized to be a "systemizing" cognitive style in males, characterized by focused learning of facts and rules of systems. Reduced testosterone in females is thought to result in a more "empathetic" cognitive style. Earlier empirical evidence to support the EMB comes from the finding that individuals with autism have a lower second to fourth finger length ratio (2D:4D); this finger length ratio is a gender dimorphic finding which has been attributed to high levels of prenatal testosterone in the male brain. In other studies to support the EMB, Baron-Cohen has reported that fT is inversely related to eye contact, vocabulary, empathy, and embedded figures performance. The most recent data to support the EMB theory were reported by Aueyung et al. (2009) who followed up a cohort of 235 children, where amniotic measures of fT were sampled in the course of clinical screening to detect genetic risk factors. When the children reached 6–10 years of age, autistic traits were assessed using parent report on standardized instruments. A positive relationship between high "autistic symptom" scores and fT was reported.

While the EMB has been referred to as the only theory of autism to provide a link between etiology, neuropsychology, and the neural basis of autism (Klin 2009), there have been several criticisms of its empirical evidence base. Skuse (2009) notes that data supporting the EMB do not make it clear whether fT actually increases the risk for a clinical diagnosis of autism, or whether fT merely reduces the threshold for detection of autistic-like symptoms. Barbeau et al. (2009) argue that the link between autism and an exaggerated male behavioral profile is problematic because individuals with autism actually do better than non-autistic individuals on some tasks considered to be measures of female traits, and which are not associated with high testosterone levels. For example, Barbeau et al. point out that the performance of a group of individuals with autism on a lexical knowledge task was more similar to the performance typically seen in females than males (Walenski et al. 2008). Barbeau et al. (2009) also argues that testosterone favors right hemisphere development, which should result in superior global processing ability; however, it is well documented that individuals with autism show a preference for processing "local" details. Barbeau et al.'s (2009) main critique of Aueyung et al.'s (2009) data linking autistic traits to fT is that the traits being measured cannot be considered "autistic since the children are not autistic, they are typical traits found in typical individuals at different levels, together with all the normal behavioral traits" (p. 27). More broadly, perhaps the most significant criticism of the EMB is the issue of testosterone levels affecting cognitive, but not physical traits in individuals with autism (Barbeau et al. 2009). There are some indirect links between testosterone-related medical conditions and females with autism; for example, Baron-Cohen et al. reported that, compared to controls, significantly more women with autism reported via a medical screening instrument a range of testosterone-linked conditions, including hirsutism, irregular menstrual cycle, polycystic ovary syndrome, severe acne, epilepsy, and a family history of

ovarian, uterine, and prostate cancers, tumors, or growths. Furthermore, significantly more mothers of females with autism reported severe acne, breast and uterine cancers, tumors, or growths, and a family history of ovarian and uterine cancers, tumors, or growths. Further research is needed to investigate whether these elevated physical and medical risks are directly related to serum levels of testosterone, and not some other hormonal risk factor.

10 Summary

This chapter highlights a new focus in the neurodevelopmental literature: gender-specific research. To paraphrase Thompson et al. (2003), "sex" is beginning to "matter" in our understanding of children with neurodevelopmental disorders, the two most common being autism and FXS. There is evidence that the clinical presentation, cognitive profile, and neurobiological trajectory which characterizes males and females with neurodevelopmental disorders are different. Our understanding of how these differences emerge is not yet clear, although biological maturation rates, sex hormones, and psychosocial factors all play a role. The clinical diagnosis, educational and mental health management plans for girls with neurodevelopmental disorders will necessarily differ from that which is currently prescribed for males, but to date there is no empirical evidence available to indicate what gender-specific management plans should entail. The findings which suggest that girls with autism have more significant fronto-striatal executive impairment and may show greater deterioration in their mental health postpuberty may flag a need for increased psychiatric/psychological review and psychoeducation for parents, leading up to this critical developmental period. The lack of gender-specific research in the neurodevelopmental disability field is perhaps in, and of itself, a contributing environmental "risk" factor for girls. To illustrate, while psychoeducation is the front-line treatment for children with neurodevelopmental disorders, current information available to parents is based on research conducted with predominately male samples, some of which is not (according to parent reports) directly applicable or salient to girls with autism. This may delay the assessment process by invoking the "wait and see" method. The lack of salient information which informs the different ways autism may manifest in girls may complicate the subsequent grieving and adjustment periods for families. Autism is often referred to as a "silent disability"; for girls, this silence equates to a greater risk for underdiagnosis, misdiagnosis, and reduced opportunities for considering gender-specific interventions.

The EMB theory is currently suggested to be the most comprehensive explanation available for the male preponderance of autism; while there is a growing body of research to support the links between fT and "autistic symptoms" in males with autism, it remains to be seen whether the clinical picture for girls with autism is really that of an "extreme male brain" or whether it is that of a "less extreme female brain." The complex pattern of gender differences for males and females affected

by autism would call for future theoretical accounts to perhaps be gender-neutral, or based on the chromosomes involved, as is the case with FXS and Skuse's X-imprinting theory. Future new discoveries about the "nature versus nurture" effects of gender in the context of neurodevelopmental disorders will have broader implications for how we understand the causes of gender differences in childhood and adolescent psychiatry.

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The Impact of Gender on Antidepressants

John J. Sramek and Neal R. Cutler

Contents

Abstract There is a large body of literature debating whether and how gender affects the metabolism, side-effect profile, and efficacy of antidepressants. Gender differences in antidepressant pharmacokinetics and efficacy profiles have been attributed to not only anatomic and physiological differences between the sexes, but also behavioral factors, comorbid disorders, and gender-specific conditions, such as pregnancy and menopause. Despite the large body of research on this topic, few definitive conclusions regarding effects of gender on antidepressant treatment exist, and much of this research is incomplete, contradictory, or not fully used to optimize the administration of antidepressants and the response to treatment. This chapter will review the latest research on gender-specific effects of antidepressant treatment, focusing on the overall, gender-related differences in efficacy, metabolism, and side-effect profile of antidepressants, and how these differences can be used to better optimize treatment of depression in a clinical setting.

Keywords Antidepressant · Depression · Efficacy · Estrogen · Female · Gender · Hormones · Male · Menopause · Metabolism · Pathogenesis · Phamacokinetics · Sex · SSRI · TCA

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1 Introduction

There is a large body of literature debating whether and how gender affects the metabolism, side-effect profile, and efficacy of antidepressants. Gender differences in antidepressant pharmacokinetic and efficacy profiles have been attributed to not only anatomic and physiological differences between the sexes, but also behavioral factors, comorbid disorders, and gender-specific conditions, such as pregnancy and menopause. For example, gender-related variance in body fat, hormone levels, liver metabolism, gastric properties, and blood flow has been shown to affect the absorption, distribution, metabolism, and elimination of a drug. In addition, behaviors such as smoking and alcohol intake, typically more prevalent among men, may also influence the pharmacokinetics of antidepressants. Pregnancy and menopause, as well as concomitant, female-specific medications, such as birth-control treatment and hormone therapy, can have an effect on the disposition and dose requirement of antidepressants. Finally, the clinical presentation, prevalence, and resiliency of the depression itself, as well as the existence of comorbid disorders such as anxiety disorder, often have gender-specific components that may influence the treatment of the disorder and the response of patients to medication.

Despite the large body of research on this topic, few definitive conclusions regarding effects of gender on antidepressant treatment exist, and much of this research is incomplete, contradictory, or not fully used to optimize the administration of antidepressants and the response to treatment. This chapter will review the latest research and literature on gender-specific effects of antidepressant treatment, focusing on the overall, gender-related differences in efficacy, metabolism, and side-effect profile of antidepressants, and how these differences can be used to better optimize treatment of depression in a clinical setting.

2 Gender Differences in the Pathogenesis of Depression

Women are at least twice as likely as men to suffer from depression (Weissman and Klerman 1977; Weissman et al. 1993). This holds true across the spectrum of depressive disorders, including atypical depression, unipolar depression, dysthymia, and seasonal affective disorder (Rapaport et al. 1995; Lucht et al. 2003; Leibenluft et al. 1995). These differences have been found in every country surveyed (Weissman et al. 1996) and across all age groups (Kessler et al. 1993). In the USA, the national lifetime risk of major depression is 21% in women versus 13% in men (Kessler et al. 1994). Greater incidence of depression in females appears to be linked to a higher first onset rate (Kessler 2003). Symptom presentation is also typically more severe in females. Depressed women are more likely to experience chronic or recurrent depression than depressed men, with an earlier age of onset and poorer quality of life (Kornstein et al. 2000b). In addition, depressed women tend to have more weight gain, anxiety, and physical symptoms than

depressed men (Frank et al. 1988; Young et al. 1990; Angst and Dobler-Mikola 1984; Williams et al. 1995), although a study of depressed adolescents found younger girls tended to lose weight (Baron and Joly 1988). Depressed women also experience more crying, guilt, and body image dissatisfaction (Wilhelm et al. 2002), while depressed men tend to experience more work inhibition, health concerns, and social withdrawal (Vredenburg et al. 1986).

The reasons for these dramatic differences between the sexes are still unknown, but many theories exist. Since gender differences in depression prevalence do not typically emerge until adolescence, studies have suggested that female's greater susceptibility may be tied to increases in the female reproductive hormones estrogen and progesterone at puberty (Nolen-Hoeksema 1990). Fluctuations in these female hormones have been suggested to make females more susceptible to both depression and anxiety (Seeman 1997), and women may experience hormonal triggers of depressive episodes tied to reproductive events (Parry 1989). In fact, numerous studies have found variations in hormonal levels tied to increases in depressive symptoms in women undergoing puberty, menstruation, and menopause (Schmidt et al. 2004; Freeman et al. 2004). In support of this theory, the postmenopausal incidence of depression in women (when reproductive hormones stabilize) was found to be similar to that of men (Bebbington et al. 2003). There is even evidence that circadian rhythms interact with reproductive hormones and confer susceptibility to depression in some women during premenstrual dysphoric disorder (PMDD), pregnancy, and the postpartum period (Parry and Newton 2001).

Differences in monamine functioning and processing may also contribute to the gender differences in the prevalence of depression. A recent study investigated tryptophan depletion in women and men who were currently in remission from a DSM-IV-defined major depressive episode. Depletion of the monoamine tryptophan produces a transient reduction in serotonin transmission and can be used for inducing depressive episodes in formerly depressed patients. This procedure triggered significantly greater depressive symptoms in women versus men, suggesting depressive vulnerability may be related to gender differences in monoaminergic function (Moreno et al. 2006). In support of this theory, positron emission tomography (PET) showed serotonin synthesis was 48% lower in females compared with males following tryptophan depletion (Nishizawa et al. 1997).

Several studies suggest that depression manifests itself with fundamentally different characteristics in males versus females. For example, young women have been found to experience more negative self-evaluation and rumination than young men, making them more susceptible to depression, and causing them to experience deeper and more prolonged depressive episodes than men (Hankin and Abramson 2001; Nolen-Hoeksema et al. 1999). In addition, changes in brain function induced by depression and antidepressants have been found to be different between the sexes. Women with a history of childhood depression exhibit midfrontal alpha wave suppression on the opposite side of the brain than men (Miller et al. 2002), and depressed women who respond to the antidepressant fluoxetine have lower purine levels compared to non-responders than men (Renshaw et al. 2001).

In addition, several neurotransmitter systems associated with mood and depression show altered levels in males and females. Here we remind the reader of the large body of evidence correlating dysfunction of the norepinephrine (Delgado and Moreno 2000; Anand and Charney 2000; Lambert et al. 2000) and serotonergic systems (Owens and Nemeroff 1994) with the pathogenesis of depression, particularly the depletion of both of these neurotransmitters. The prolongation of serotonin (5-HT) and norepinephrine (NE) in the synaptic cleft is the mechanism of action for two major classes of antidepressants, appropriately named selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). Different concentrations of serotonin and its metabolites have been found in the brains of same-aged males and females (Legato 1997). Baseline serotonin production was found to be 52% higher in the brains of young adult men compared to young adult women when measured with a PET radioligand (Nishizawa et al. 1997), and this was supported by another recent PET study (Sakai et al. 2006). Older, postmenopausal women were found to have lower levels of serotonin and cortisol than older men (Young 1995), and a percentage of depressed women showed lower-than-normal norepinephrine activity for their age (Halbreich and Lumley 1993). Serotonin metabolism and the serotonin metabolite 5HIAA were found to be elevated in females versus males (Young et al. 1980), possibly related to higher availability of the serotonin transporter 5-HTT in women (Staley et al. 2001). In addition, the serotonin 5-HT_{1A} receptor subtype, which is implicated in the pathogenesis of depression, shows a significant decrease in binding potential with age in males but not females (Meltzer et al. 2001). Brain levels of serotonin and norepinephrine show greater age-related changes in females compared to males (Legato 1997). Platelet 5-HT and levels of the serotonin metabolite 5-HIAA were significantly higher in older women compared to younger women (89.41 ng/108 platelet for younger vs. 112.9 for older; 1.20 for younger vs. 2.19 for older, respectively) (Kumar et al. 1998).

The dopaminergic system has also been found to play a role in the pathogenesis of depression, and a large body of recent literature has focused on altering brain levels of dopamine (DA) as a therapeutic intervention in depression. DA turnover has been shown to be reduced in depressed patients (Brown and Gershon 1993), and chronic antidepressant treatment can alter DA transmission (Dhir and Kulkarni 2007). Homovanilic acid (HVA), a major metabolite of DA, has been found to be reduced in the CSF (Roy et al. 1985; Hamner and Diamond 1996) and plasma (Lambert et al. 2000) of depressed patients. Neuroimaging and histopathological studies have found D2/D3-receptor binding to be increased, and dopamine transporter (DAT) binding to be decreased, in patients suffering from major depressive disorder (MDD) in comparison to healthy controls (Klimek et al. 2002; D'Haenen and Bossuyt 1994; Shah et al. 1997; Meyer et al. 2001). DA may also promote neurotrophic processes in the adult hippocampus (Guiard et al. 2009), and is the target for new classes of antidepressants, such as triple monoamine reuptake inhibitors and dopamine agonists (Rakofsky et al. 2009).

Gender effects appear to play a role in the dopaminergic system as well. Neuroimaging studies have suggested that women have a higher synaptic

concentration of dopamine in the striatum than men. They also show that women have a higher striatal dopamine synthesis capacity than men, and that age decreases this capacity in men more than in women (Laakso et al. 2002). Preclinical studies have suggested that female sex hormones enhance presynaptic dopamine turnover (Shimizu and Bray 1993; Xiao and Becker 1994). Women experience reduced striatal dopamine release in response to amphetamines than men (Munro et al. 2006). In addition, women in the luteal phase of the menstrual cycle, which is associated with high levels of progesterone, display reduced responses to amphetamine and cocaine (inhibitors of DA reuptake) compared to men (Sofuoglu et al. 2004; White et al. 2002). Females have been shown to exhibit higher levels of DAT activity than males (Dluzen and McDermott 2008), supported by preclinical studies suggesting higher DAT density in female rats versus male rats (Rivest et al. 1995). In contrast, male rats exhibit higher production and turnover of the D1 and D2 dopamine receptors (although both genders show comparable receptor density) (Andersen et al. 1997).

While numerous and varied gender differences have been reported in the serotonergic, dopaminergic, and norepinephrine systems, the significance of these changes, and how they interact to increase or decrease the susceptibility of each gender to depression, requires further study.

Depression in females may also correlate with reductions in reproductive hormones due to menopause. Surgical menopause has also been found to increase depressive symptoms (Shifren and Avis 2007). In addition, oophorectomized females who received estrogen replacement therapy (ERT) reported diminished anxiety and depression compared to oophorectomized females not receiving ERT (Nathorst-Boos et al. 1993). While suggestive of a palliative role for estrogen in the treatment of depression, the evidence for this remains ambiguous, as other studies have not shown postmenopausal women to be at an increased risk for depression (Winokur 1973).

3 Gender Differences in Response to Antidepressants

Despite decades of research on sex-related differences in antidepressant treatment, the consensus is still out on whether such a difference exists, and if so, whether it is dependent on the class of antidepressant, menopausal state, BMI index, hormonal interactions, or PD/PK characteristics, among other variables. There are numerous studies that have shown significant gender differences in regards to the efficacy of certain antidepressants (see Table 1 for a list of studies showing gender effects). For example, several studies have found a significantly greater therapeutic response among men compared to women after taking the tricyclic antidepressant (TCA) imipramine (Dawkins and Potter 1991; Hamilton et al. 1996; Kornstein et al. 2000a; Frank et al. 1988). Another study found that women had more therapeutic benefit with the SSRI sertraline versus the TCA imipramine, whereas men responded similarly to both antidepressants

Table 1 Studies finding gender-based efficacy differences with antidepressants

Reference	Drug type	Study type	Subjects	Results
Haykal and Akiskal (1999)	SSRIs, TCAs	TCA-type antidepressants or fluoxetine	25 male and 17 female primary dysthymic patients	Females responded better than males to SSRIs
Kornstein et al. (2000a, b)	SSRIs, TCAs	12-week double-blind trial with sertraline or imipramine	235 male and 400 female outpatients with chronic major depression or double depression	Females responded better to SSRI sertraline, males responded better to TCA imipramine; differences observed primarily in premenopausal females
Martenyi et al. (2001)	SSRIs, TCAs	6-week, double-blind trial of SSRI (fluoxetine) and a norepinephrinergic TCA (maprotiline)	105 male and female depressed patients	Females in their reproductive period were more responsive to SSRIs than norepinephrinergic TCAs
Quitkin et al. (2001)	TCAs, MAOIs, SSRIs	20-year review of 8 placebo-controlled antidepressant trials and 1 open-label study	1,746 depressed patients between 18 and 65	Older females had superior response to TCAs than younger females; females had statistically superior response to MAOIs than males
Khan et al. (2005)	SSRIs, SNRIS	Review of 15 randomized, placebo-controlled trials for sex differences in antidepressant efficacy	323 depressed patients	Females had a significantly greater response than males to SSRI and (to a lesser extent) SNRI treatment
Berlanga and Flores-Ramos (2006)	SSRIs, SNRIs	8-week, double-blind clinical trial for gender differences in SSRI citalopram and SNRI reboxetine	86 depressed patients (48 females, 38 males) 18–40 years old	Premenopausal females respond better than males to serotonergic antidepressants
Young et al. (2009)	SSRI	12–14-week study of citalopram	1,043 male and 1,833 female patients with single or recurrent nonpsychotic MDD	Females have a better response to the SSRI citalopram than males

(Baca et al. 2004). In addition, a study of atypical depression found monoamine oxidase inhibitors (MAOIs) were superior to TCAs in women, while TCAs were superior to MAOIs in men (Davidson and Pelton 1986). Several studies also suggest women may respond better to SSRIs than men (Kornstein et al. 2000a; Thase et al. 2000; Martenyi et al. 2001; Haykal and Akiskal 1999; Khan et al. 2005; Sagud et al. 2002; Young et al. 2009), although the results of one of these studies has been disputed (Quitkin et al. 2001). A recent study found that younger women exposed to the SSRI fluvoxamine not only had a better response than men, but also a better response than older women (>44 years old) (Naito et al. 2007).

In contrast, there are a large number of studies that have detected no gender differences in regards to the efficacy of various antidepressants, even when examining the same class of antidepressants as the studies which found gender differences (see Table 2 for a list of studies showing no gender effects). For example, a study found the serotonin-norepinephrine reuptake inhibitor (SNRI) venlafaxine and SSRIs produced a comparable response in both men and women (Entsuah et al. 2001). A large, retrospective study of TCAs, MAOIs, and the SSRI fluoxetine found no difference based on sex or menopausal status on drug efficacy (Quitkin et al. 2002), and additional studies found women did not respond preferentially to SSRIs (Thiels et al. 2005), nor did men respond preferentially to TCAs (Parker et al. 2003). Adding fuel to this argument, Hildebrandt et al. (2003) found no effect of gender on therapeutic outcome in patients treated with the TCA clomipramine, nor with the SSRIs citalopram and paroxetine, nor with the MAOI moclobemide. A meta-analysis of 30 randomized placebo-controlled trials of imipramine or amitriptyline also found no effect of gender on TCA efficacy(Wohlfarth et al. 2004).

While it is unclear why such contradictory data exist, there are numerous methodological issues which could explain such differences. For one thing, these studies did not all use the same criteria for determining what constituted a significant response to treatment. For example, one study used a paired *t*-test to compare total HAMD17 baseline to posttreatment scores (Martenyi et al. 2001); another study required a 50% or greater decrease in HAMD21 scores to indicate a significant response to treatment (Entsuah et al. 2001). In addition, the varied ages of the female patients in these studies could also play a role, as older, postmenopausal women have very different levels of female sex hormones than younger, premenopausal women, and these hormones may affect antidepressant efficacy and metabolism. Other potential sources of variability include the clinical presentation of the depressed patients (i.e., typical versus atypical), whether the patient was treated with antidepressants previously, the various drugs and doses used across studies, and how well the patient adhered to the treatment regimen. In addition, some studies were prospective, while others were a meta-analysis of large data pools. Better-controlled studies are needed to account for these numerous variables and tease out any influence of gender on antidepressant efficacy.

Table 2 Studies finding no gender-based efficacy differences with antidepressants

Reference	Drug type	Study type	Subjects	Results
Entsuah et al. (2001)	SSRIs, SNRIs	Meta-analysis of 8 comparable double-blind, active-controlled, randomized SSRI or venlafaxine clinical trials	2,045 patients with major depression or major depressive disorder, aged 18–83 years	Males and females have comparable responses to SSRIs and SNRIs across various age groups
Hildebrandt et al. (2003)	SSRIs, TCAs, MAOIs	Review of 3 Danish double-blind randomized, controlled trials	292 inpatients (96 males, 196 females) with major and predominantly melancholic depression	No relationship between plasma concentrations, gender, and therapeutic outcome
Wohlfarth et al. (2004)	TCAs	Review of 30 randomized, placebo-controlled trials of antidepressant efficacy	3,886 patients (1,555 males and 2,331 females) with depression	Tricyclic antidepressant response is independent of gender
Thiels et al. (2005)	SSRIs	Review of data from a 6-month prospective sertraline utilization observation study	1,594 male and 3,858 female depressed patients	No gender difference in side-effects, treatment termination, or treatment response to SSRI
Pinto-Meza et al. (2006)	SSRIs	6-month follow-up study of antidepressant treatment with a SSRI (citalopram, fluoxetine, paroxetine, or sertraline)	242 females (95 in their menopause) and 59 males with major depression	No gender differences were observed in treatment response, depression severity, and symptomatology

3.1 Pharmacokinetic Differences

Women and men have been found to exhibit different pharmacokinetic (PK) profiles of antidepressants. Different PK profiles stem from many different sources, including gender-related variance in body weight, plasma volume, gastric acid production, gastric emptying time, plasma protein levels, enzyme activity, drug transporter function, and drug clearance rates, among others. Women have been shown to have higher plasma levels (Hamilton et al. 1996; Preskorn and Mac 1985) and lower clearance of TCAs (Gex-Fabry et al. 1990). This may partially be attributed to the fact that women have a higher percentage of adipose tissue and body fat compared to men. Antidepressants are lipophilic and have a strong affinity for adipose tissue, typically resulting in a greater volume of drug distribution in women (Yonkers and Brawman-Mintzer 2002; Yonkers et al. 1992). Lipophilic drugs tend to have a greater distribution in women, while water soluble drugs tend to have a greater distribution in men. In addition, women tend to secrete less gastric acid and empty the contents of their stomach more slowly than men, and have slower gastric motility in the presence of female sex hormones, resulting in reduced breakdown and clearance of antidepressants (Hutson et al. 1989; Young et al. 2009).

A review study summarized data supporting sex differences in the activity of various antidepressant-metabolizing enzymes, although little research exists into how these differences might translate into differences in clinical efficacy (Yonkers and Brawman-Mintzer 2002). Several studies found lower levels of the drug transport protein P-glycoprotein in females, which could result in altered bioavailability of certain drugs in women (Lan et al. 2000; Schuetz et al. 1995). These differences were not observed in a subsequent study, suggesting that further investigation of this transport protein is necessary (Kim et al. 2001). Studies have also found that estrogen affects the binding of metabolic enzymes to certain glycoproteins, which could potentially affect drug metabolism if those drugs were composed of glycoproteins or glycoprotein homologues (Succari et al. 1990).

The cytochrome P450 (CYP450) enzyme superfamily is one of the major drug metabolizing systems in humans, and several of its constituent parts exhibit gender differences which could contribute to observed gender differences in the PK profile of antidepressants. The metabolic enzyme CYP3A4 is highly expressed in the liver and has the largest range of substrates of all CYP enzymes, making it one of the most important enzymes for breaking down xenobiotics. It plays a major role in the metabolism of many of the drugs taken into the body, including numerous SSRIs (including citalopram, escitalopram, fluoxetine, and sertraline) and TCAs (including amitriptyline, imipramine, and clomipramine). Drugs that are substrates of CYP3A4 have often been found to clear faster in women than in men (Meibohm et al. 2002), which may be due to higher observed CYP3A4 enzymatic activity in women than in men (Hunt et al. 1992; Schmidt et al. 2001). On the other hand, drugs that are substrates of CYP2D6, another major metabolizer of xenobiotics, including the TCAs desipramine (Abernethy et al. 1985) and mirtazapine (Timmer et al. 2000),

have often been shown to clear faster in men than in women (Labbe et al. 2000). Substrates of CYP1A2 have generally been found to clear faster in men than in women (Ou-Yang et al. 2000; Ereshefsky et al. 1991; Bruno et al. 1997), although there are studies that have shown the exact opposite (Nafziger and Bertino 1989). In addition, studies have shown that substrates of CYP2E1 also clear faster in men than in women (Lucas et al. 1995; Kim and O'Shea 1995).

In addition to the CYP450 system, there are many other metabolic enzymes that display gender differences in function, and these enzymes also contribute to the PK profile of xenobiotics. For example, substrates of the enzymes thiopurine methyl transferase, glucuronidation, dihydropyrimidine dehydrogenase, UDP-gluronosyl transferase, and catechol-*O*-methyl transferase all show greater clearance in men than in women (Franconi et al. 2007). On the other hand, substrates of xanthine oxidase show greater clearance in women than in men (Bock et al. 1994).

Determining how these numerous (and often contradictory) gender-based differences in metabolic enzymes and functions combine to affect the breakdown and distribution of antidepressants is an extremely complex task. It remains to be determined exactly what the clinical significance these differences are in regards to antidepressant efficacy. Most likely this effect will vary not only by the class of antidepressant, but also by the individual structure of each unique antidepressant compound.

3.2 Female Reproductive Hormones

Levels of estrogen and other female reproductive hormones have been suggested to play a role in the pathogenesis of depression and the efficacy of antidepressants. This has been evidenced, in part, by the difference in susceptibility to depression, and the difference in response to antidepressants, in women before and after menopause – an event associated with a dramatic drop in reproductive hormone levels. Premenopausal women respond better to certain classes of antidepressants than postmenopausal women. Likewise, depressed, postmenopausal women receiving ERT in combination with a SSRI showed better clinical response than depressed, postmenopausal women receiving the SSRI alone (Schneider et al. 1997). A 6-week, open-label, naturalistic study looked at the response of premenopausal and postmenopausal women with MDD to various antidepressants, including SSRIs, SNRIs, and TCAs. The study found that postmenopausal status predicted a poor response to antidepressants, and this also correlated with high levels of follicle-stimulating hormone (FSH) (Pae et al. 2009). Several studies have found both FSH and luteinizing hormone (LH) to be elevated in postmenopausal depression (Harlow et al. 2003; Abe et al. 1985; Freeman et al. 2006). Supporting this finding, improvements in depressive symptoms in peri-menopausal women have been correlated with decreases in FSH levels (Daly et al. 2003). Levels of estradiol (E2) have also been reported lowered in depressed premenopausal and peri-menopausal women (Harlow et al. 2003; Young et al. 2000).

Female reproductive hormones have effects that may interfere with or enhance the efficacy of antidepressants. For example, progesterone and estrogen have been found to modulate neurotransmitter synthesis, release, and reuptake – characteristics that many antidepressants also modulate (Frackiewicz et al. 2000). Estrogen has also been found to increase new dendritic spine formation and regulate neurotropic factors (Bryant et al. 2006). In addition, progesterone in the luteal phase has been found to slow gastric emptying, potentially modifying an antidepressant's pharmacokinetic properties (Yonkers et al. 1992). Estrogen has been found to interact with the serotonergic system, which is the target of many antidepressants, including SSRIs and SNRIs. Serotonergic agents have been found to be more potent in the presence of estrogen (Halbreich et al. 1995). In addition, the serotonin releaser, fenfluramine, had a greater effect on postmenopausal women taking ERT than ERT-naive, postmenopausal women (van Amelsvoort et al. 2001).

3.3 The Role of Menopause

Studies looking at menopause and antidepressants tend to support the theory that estrogen and other reproductive hormones enhance antidepressant efficacy. For example, a recent study looked at 242 depressed women (95 in menopause), and 59 depressed men beginning antidepressant treatment with an SSRI (citalopram, fluoxetine, paroxetine, or sertraline) from 16 primary care centers over the course of 6 months. The study found that menopause, characterized by significantly reduced female reproductive hormones (including estrogen), seems to negatively affect the SSRI treatment response of depressed women (Pinto-Meza et al. 2006). Another study examined 115 female outpatients with MDD (divided into premenopausal and postmenopausal status), as well as 86 age-matched male outpatients, before and after 8 weeks of treatment with the SSRI nefazodone or venlafaxine. The study found that women are more responsive to SSRIs during their reproductive period than during their menopausal period (Grigoriadis et al. 2003). Another study looked at the response of 86 depressed male and premenopausal female patients (18–40 years old) to the SSRI citalopram and the serotonin-norepinephrine reuptake inhibitors (SNRI) reboxetine, in an 8-week, double-blind clinical trial. The study concluded that premenopausal women respond better than men to serotonergic antidepressants, further suggesting that female gonadal hormones such as estrogen may have an enhancing effect on antidepressant efficacy (Berlanga and Flores-Ramos 2006).

These results are far from conclusive, as estrogen may function as a general mood-enhancer, independent of its hypothesized role as an antidepressants-enhancer. Estrogen given to peri-menopausal women not on antidepressants was found to be an effective treatment for depression (Soares et al. 2001). In addition, abrupt interruption of ERT in women aged over 40 with recurrent depression can quickly induce a new depressive episode (Stewart et al. 2004). On the other hand, numerous studies have not found a higher risk for depression in women

during their postmenopause phase, when reproductive hormones drop significantly (McKinlay et al. 1987; Avis et al. 1994). In support of this, another study found that estrogen alone was ineffective at relieving depression in most postmenopausal women (Morrison et al. 2004).

Low LH levels also correlate and may be predictive of better antidepressant response in postmenopausal women (Zanardi et al. 2007). Serotonin levels have been found to be inversely correlated with LH levels (Carretti et al. 2005). Since low LH levels imply higher serotonin levels, this may provide a more potent substrate for antidepressants whose mechanism of efficacy is dependent upon the availability of serotonin (such as SSRIs and SNRIs). Low LH levels also correlate to hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis (Vadakkadath Meethal and Atwood 2005; Swaab et al. 2005), which represents the complex interactions between these organs that is essential in mood regulation, and is disrupted in MDD (Pariante 2003). As antidepressants typically work by reducing HPA activity, patients with low LH levels may have a hyperactive HPA axis, and are therefore good candidates for experiencing symptom improvement via antidepressant treatment (Holsboer and Barden 1996; Holsboer 2000).

The menstrual cycle also may play a role in attenuating antidepressant efficacy. The menstrual cycle can affect gastric motility, as fluid retention may result in diluted plasma levels of a drug (Yonkers et al. 1992). In addition, fluctuating levels of female sex hormones during the menstrual cycle may interact in complex ways with antidepressant metabolism. All of these factors contribute to the complex pharmacokinetic profile of antidepressants in women. More research is required to understand how each of these factors work together to produce observed gender differences in the efficacy of many antidepressants.

4 Conclusion

Despite decades of research into the effect of gender on depression pathogenesis and antidepressant efficacy, the consensus is still out on what these effects are, how these effects are produced, and in some cases, whether these effects exist at all. While many studies have found a higher incidence of depression in females, with a different array of symptoms and severity of symptom presentation, the reasons for these differences remain unknown. Likely, female sex hormones are at least partially involved, as periods of fluctuating levels of estrogen and progesterone have been linked to increased incidence of depression, such as during puberty and the transition to menopause. Differences in monoamine function, neurotransmitter metabolism, and even innate brain structures may also play a role in depression susceptibility, as numerous studies have identified an array of gender-specific differences in structures implicated in the pathogenesis of depression.

These systems are thought to play a role in producing gender-specific differences in antidepressant therapy, although their discrete role is far from clear, and even whether such gender differences exist remains controversial. There are almost an

equal number of published studies showing gender differences, and no gender differences, in the efficacy profile of identical classes of antidepressants. This contradiction may be due to numerous factors, including methodological differences between studies in the measurement of a statistically significant treatment response.

A better consensus exists regarding gender differences in antidepressant metabolism. Sex-specific variance has been identified in numerous pharmacokinetic components, including plasma volume, gastric acid production, gastric emptying time, plasma protein levels, metabolic enzyme activity, drug transporter function, and drug clearance rates, among others. In addition, a large body of literature exists examining the effects of female hormones on the efficacy of antidepressants. For example, numerous studies suggest that estrogen has an enhancing effect on antidepressant efficacy, as evidenced by the poorer response of postmenopausal women to a variety of antidepressants when compared with premenopausal controls. This finding has direct therapeutic implications for treating depressed, postmenopausal women. On the other hand, lower levels of LH have been correlated with improved antidepressant efficacy.

Most likely it is the delicate interplay between all of these complex, multivariate systems that produces the observed gender differences in depression susceptibility and treatment response. More studies are required to tease apart each of these factors to understand how they contribute to the disease phenotype and the efficacy of antidepressants.

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