

**Estrogens, Brain and Behavior:
Lessons from mouse models**

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Abstract

The use of animal models to effectively replicate problems such as hormone deficiencies, neurological diseases, and brain injury and stroke, has certainly made a vast contribution to understanding the neuroprotective effects of estrogen in the brain. Studies using ovariectomy procedures followed by 17β -estradiol replacement have effectively demonstrated the positive effects that estrogen provides in cognitive performance and memory performance tasks. The majority of rodent models have analyzed the physiological effects of estrogen by methods of ovariectomy followed by placebo or 17β -estradiol replacement. A major problem with such studies is that local brain aromatase (the estrogen-synthesising enzyme) may still convert circulating androgens to estrogens. Hence, such 'estrogen deficient' model may not be completely void of estrogen. The generation of the aromatase knockout (ArKO) mouse and the estrogen receptor knockout (ERKO) mice has enabled researches to characterize the complete lack of estrogen effects within the brain. This review aims to compare and contrast the results of these various mouse models.

Introduction

Sex hormones exert powerful influences on both normal and pathological behavior and may modulate the risk of neuronal death in neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). Aromatase is expressed in the brain of both sexes, although gonads are the major sites of expression. As a consequence, it is difficult to study effects of estrogens in the brain and on behavior because it is synthesised locally in the brain and gonadectomy will not remove its effects. The aromatase knockout (ArKO) mouse has total estrogen deficiency, even in the brain. Interestingly, it presents sexually dimorphic brain or behavior phenotypes. This animal model is a useful tool to study the reasons that sex hormones make some neurones more susceptible to death and provide insights into the mechanisms of compulsive behavior, especially when its estrogen receptors (ERs) are totally responsive to administered estrogens or ER-specific agonist.

NEUROPROTECTIVE EFFECTS OF ESTROGENS

The presence of aromatase and ER mRNA and protein expression in several regions of the brain suggests a functional role for estrogen in the brain. Indeed, several reports have demonstrated the neuroprotective effects of estrogen in the brain. Such neuroprotective actions of estrogens have been implicated in the treatment of cognitive and memory dysfunctions, brain injury and stroke (Azcoitia et al 2001; Wise et al 2001b), neurodegenerative diseases such as Alzheimer's Disease (Tang et al 1996; Xu et al 1998), and Parkinson's disease (Cyr et al 2002). The mechanisms of estrogens' neuroprotective actions are still questionable, although several different modes of action have been suggested in preventing and relieving insult to the brain, such as anti-apoptotic effects, protection from free radicals, anti-inflammatory effects, regulation of calcium channels and

protection via increasing cerebral blood flow, (see review (Amantea et al 2005). The majority of these effects are mediated through estrogen receptor activation, and in addition rapid, non-genomic actions appear to be regulated by the newly identified membrane bound estrogen receptor, (see review (Amantea et al 2005). Other non-genomic effects appear to be due to the antioxidant free-radical scavenging properties of the estrogen steroid structure, (see review (Wise et al 2001b). Neuroprotective effects of estrogen have been explored using several different models and techniques from mouse models of induced brain injuries to clinical studies and *in vitro* cell culture analysis. Below the wide range of estrogens' neuroprotective properties are discussed in detail.

Clinical and epidemiological studies on the neuroprotective effects of estrogen

A large body of evidence is mounting which strongly advocates estrogen as a neuroprotective agent in the prevention and, in some instances amelioration, of neurological and mental disorders. Clinical trials of treatment with 17 β -estradiol, for women diagnosed with schizophrenia has demonstrated that estradiol may have antipsychotic properties, or act as a catalyst for neuroleptic responsiveness. Another potential mechanism suggested from the beneficial effects of estradiol treatment was that estrogen may be involved in modulation of dopaminergic activity in schizophrenic patients, (see review (Cyr et al 2002). Depletion of dopaminergic synthesizing neurons within the substantia nigra pars compacta is the main characteristic of Parkinson's disease, a disorder which leads to progressive motor dysfunction. Parkinson's disease is more prevalent in men than in women and in addition symptoms evolve quicker and men are less responsive to levodopa treatment than women, (see review (Cyr et al 2002). These differences may be attributed to the level of steroid hormones such as testosterone and local estrogen levels. Whilst some studies have demonstrated that estrogens improve motor disability in post-menopausal women with Parkinson's

disease (Tsang et al 2000), others have found no effect of estrogen therapy (Blanchet et al 1999; Strijks et al 1999), (see review (Cyr et al 2002)). As well as dopaminergic cell loss, Parkinson's degeneration also involves oxidative stress, excitotoxicity and metabolic challenges, and estrogen may exert protective properties through disruption of many of these degenerative processes, (see review (Cyr et al 2002)).

Estrogens have also been reported to decrease the incidence and delay the onset of Alzheimer's disease, however this is a point of great debate. Alzheimer's disease (AD) is more prevalent in women than in men, and the incidence of AD increases with age, rising from 1.2 per 1000 at age 65 to 63.5 per 1000 by age 90, (see review (Henderson 2006)). This age related increase coincides with the menopause in women, and therefore suggests a role for steroid hormones in protection against AD, (see review (Henderson 2006)). Whilst early clinical studies on hormone replacement therapy suggested that Alzheimer's disease (AD) women receiving hormone replacement therapy (including estrogen supplements) have milder symptoms of dementia compared with women with AD not using hormone replacement therapy (Doraiswamy et al 1997; Henderson et al 1996) . Thereafter, several small, short term trials reported positive findings of estrogen therapy in AD patients. However, several larger trials involving greater numbers and longer treatment periods reported no difference in cognitive or functional outcomes with AD women on estrogen compared to without, (see review (Henderson 2006)). Several explanations have been proposed for such discrepancies, suggesting that the type of progestogen used, and the time of estrogen therapy initiation may be causative factors. Indeed, studies have shown quite convincingly that early in menopause, when neurons are still in a healthy state estrogen therapy can be quite effective, however once the disease has progressed and is manifested in the neurons, estrogen treatment appears to be ineffective (see review (Amantea et al 2005)).

Whilst several studies have been conducted on estrogens' neuroprotective effects in neurodegenerative disease, more recently estrogen has been shown to possess strong neuroprotective properties following brain injury or stroke. Evidence first arose when several studies found that females were less vulnerable to acute brain injuries following cerebral ischemia, hypoxia and drug induced toxicity (see review (Wise et al 2001b)). The incidence of cerebrovascular stroke in premenopausal women is significantly lower than men, however this sex difference is ameliorated in post-menopausal women when compared to age matched men, (see review (Wise et al 2001b)). This suggested a strong case for estrogen in the prevention of stroke and/or traumatic brain injury, and indeed, several studies proved that estrogen treatment diminished the extent of injury, (see review (Wise et al 2001b)). In addition, continued use of estrogens has been demonstrated to reduce the risk of stroke by 50% (see review (Amantea et al 2005)).

In vitro studies on the neuroprotective effects of estrogen

Several studies have used neuronal cell cultures to demonstrate the neuroprotective actions of estrogen. These neuroprotective effects range from stimulating neuronal outgrowth and dendritic branching, antioxidant effects, and anti-apoptotic effects. A wide range of studies have demonstrated that 17 β -estradiol treatment enhances cell survival in a variety of cell lines including hypothalamic (Duenas et al 1996), amygdaloid (Arimatsu and Hatanaka 1986) and cortical, although whilst in parietal and occipital cortical neurons estradiol has a significant effect on neuron outgrowth, this effect was not significant in neurons from the frontal cortex and estradiol actually decreased neuron outgrowth in the temporal cortex (Brinton et al 1997). This indicates regional differences in estradiol mediated actions within the cortex. A more recent study identified that ER β is essential in estrogen mediated effects of migration and neuronal survival in the cerebral cortex (Wang et al 2003). 17 β -

estradiol treatment also proved effective in facilitating survival and process extension of neurons from cultured hippocampal cells at concentration of 1-100ng.ml. Furthermore, at higher concentration of 100microg/ml estradiol treatment led to scavenging of free radicals (Sudo et al 1997), and thus stresses the different mechanisms of estrogen action depending on concentrations administered. This study was confirmed by more recent work which demonstrated that 10nM of 17 β -estradiol effectively stimulated dendritic branching, and this effect was not blocked by the ER antagonist ICI 182, 780, indicating no involvement of the classic ER pathway (Audesirk et al 2003).

As well as the stimulatory effects, estrogens have been shown to possess neuroprotective properties following neuronal toxicity. Several studies have demonstrated that 17 β -estradiol treatment prior to exposure to the excitatory neurotransmitter, glutamate, significantly reduces neuronal death in cultures of hippocampal, ventral mesencephalon, and cortical neurons (Huang et al 2004; Sawada et al 1998; Singer et al 1996; Sribnick et al 2004; Zaulyanov et al 1999). In all of these cases pretreatment before induced glutamate toxicity was effective in preventing cell death, conversely simultaneous treatment administered at the same time as the glutamate injection was not effective (Sawada et al 1998). Whilst some studies reported that this neuroprotective effect was blocked by ER antagonist ICI 182 780, or anti-estrogen tamoxifen, indicating classic ER mediated actions(Huang et al 2004; Singer et al 1996), others reported that these effects were more likely to be a membrane ER mediated action as the effects were very rapid, and in addition 17 α -estradiol also showed neuroprotection, and this form cannot bind to the classic ERs (Sawada et al 1998; Zaulyanov et al 1999). Pretreatment with 17 β -estradiol also neuroprotects against toxicity induced by superoxide anion and hydrogen peroxide (Sawada et al 1998; Singer et al 1996), pro-oxidant hemoglobin (Regan and Guo 1997), and NMDA-induced neuronal death (Weaver et al 1997).

The large number of studies indicating the positive effects of estrogen following neuronal toxicity led researches into the possibility of neuroprotection afforded by estrogen in neurodegenerative disease processes. Indeed estrogens have been shown to protect primary hippocampal and cortical neurons in vitro from the destructive effects of the HIV envelope glycoprotein gp120, and this effect of estrogen is reported to be an antioxidant action as estrogen protection here occurred independent of ER binding (Brooke et al 1997; Howard et al 2001). Several studies have found that pretreatment with 17β -estradiol prior to exposure to amyloid beta peptide, a neurotoxic peptide found in Alzheimer's disease (AD), protected neurons in a significant manner (Bonfont et al 1998; Goodman et al 1996; Hosoda et al 2001; Marin et al 2003; Moosmann and Behl 1999; Zhang et al 2004b). However, whilst some explain estrogens neuroprotective actions here by antioxidant effects (Goodman et al 1996; Moosmann and Behl 1999), others describe the neuroprotection afforded by estrogen in amyloid beta induced toxicity to be an anti-apoptotic, and ER-mediated effect (Hosoda et al 2001; Marin et al 2003; Zhang et al 2004b). Estradiol pretreatment has also been shown to be efficient in neuroprotection against the cytotoxic effects of 1-Methyl-4-phenyl pyridium MPP⁺ using neuronal PC12 cells in a cell culture model of Parkinson's disease (Gelinas and Martinoli 2002). In addition 17β -estradiol treatment restored dopamine transporter protein expression levels in MPP⁺ treated cultures to control levels (Gelinas and Martinoli 2002). Taken together, these in vitro studies demonstrate that the 17β -estradiol has strong neuroprotective properties. However, several key factors need to be taken into consideration when analyzing these neuroprotective actions. Firstly, the majority of these studies only found significant effects with pretreatment of 17β -estradiol before the onset of neuronal toxicity. Secondly, the dose of 17β -estradiol administered seems to play a role in the effectiveness of the treatment, and the mechanism

of action (i.e. rapid membrane effects, or slower genomic effects). Finally, the specific brain region in question may react differently to 17β -estradiol treatment.

In vivo studies on the neuroprotective effects of estrogen

Rat and mouse models of ischemic brain injury and stroke, produced by middle cerebral artery occlusion (MCAO) have shown a higher recovery rate and significantly reduced brain damage following 17β -estradiol treatment, and this result was effective using both pharmacological and physiological 17β -estradiol concentrations, (see review (Wise et al 2001a). This effect was region specific as whilst 17β -estradiol treatment reduced cerebral and hippocampal infarct damage, it had no effect on the striatum following MCAO (Merchenthaler et al 2003; Wise et al 2001a). Furthermore, the female ArKO mice demonstrated greater ischemic damage after MCAO when compared to WT controls (McCullough et al 2003), confirming that neuroprotective effects of estrogens.

Neuroprotective effects of estrogens in MCAO animal models have been reported to be mediated via $ER\alpha$ only in the hippocampus, and via activation of both ER subtypes in the subventricular zone (Merchenthaler et al 2003; Suzuki et al 2007). More recently it was demonstrated that $ER\alpha$ is induced in the early stages of MCAO injury, whilst $ER\beta$ plays a role in the later stages (Dubal et al 2006). Mechanisms of neuroprotection afforded by estrogen in MCAO models include anti-apoptotic regulation of bcl-2 expression, and anti-inflammatory effects via decreasing levels of the inducible isoform of nitric oxide synthase (iNOS) (Alkayed et al 2001; Park et al 2006).

Models of brain injury and assault involving administration of kainic acid (a neurotoxin), have demonstrated that high doses of 17β -estradiol can protect the brain from neuronal damage induced by kainic acid injections (Picazo et al 2003). Pretreatment with 17β -estradiol for one week prior to kainic acid injection significantly reduces neuronal cell death (Wilson et al 2000). In addition, the estrogen deficient model, aromatase knockout (ArKO) mice showed significant neuronal loss in the hilus of the hippocampal after injection of neurotoxin domoic acid at a dosage that had no effects on the wildtype littermates (Azcoitia et al 2001), indicating that aromatase deficiency increases the vulnerability of hilar neurons to neurotoxic degeneration. Hill et al (2004) also showed that the dopaminergic neurons in the 1 year-old male ArKO undergo apoptosis without external assault. This Fas/FasL-mediated apoptosis in the arcuate nucleus and medial preoptic area can be prevented by selective ER α and ER β agonist respectively (Hil et al 2007) which mirrors the abundance of the ER subtypes in these regions (Mitra et al).

Mouse models of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease have also found beneficial effects upon 17β -estradiol treatment. Parkinson's disease mouse models have been created by inducing lesions within the striatal region via injection of 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). Sex differences in the neurotoxic effects of lesioning have been shown, with males displaying more dramatic dopaminergic cell loss than females, indicating involvement of sex steroid hormones, (see review (Cyr et al 2002)). Indeed, 17β -estradiol treatment reduced the increased level of glial fibrillary acid protein (GFAP) which occurs due to induced MPTP lesions, (see review (Cyr et al 2002)). Furthermore 17β -estradiol treatment has demonstrated rapid, dose-dependent increases in dopaminergic neuron activity within the striatal region of ovariectomized rats, (see review (Cyr et al 2002)). In concur with these observations,

female ArKO mice exhibited a far greater vulnerability to MPTP-induced nigrostriatal damage when treated with MPTP, as compared to their wildtype gonadally intact and gonadectomized counterparts (Morale, 2008). Furthermore, aromatase deficiency from early embryonic life significantly impairs the functional integrity of SNpc tyrosine hydroxylase-positive neurons and dopamine transporter innervation of the caudate-putamen in adulthood (Morale, 2008).

Several discrepancies in the literature have arisen over the protective effects of estrogen found in mouse models replicating Alzheimer's disease. The extracellular deposition of amyloid peptides in plaques and neurofibrillary tangles are the two characteristics in the pathology of Alzheimer's disease (AD) (Levin-Allerhand et al 2002). Mutant β -amyloid protein precursor transgenic mice, ovariectomised and treated with placebo or 17α - or 17β -estradiol showed decreased β -amyloid levels by 27% and 38% in 17β - and 17α -estradiol treated mice respectively (Levin-Allerhand et al 2002). Furthermore this β -amyloid lowering effect was replicated with lower physiological levels of 17α - and 17β -estradiol applied in the drinking water, and thus presents a strong case for the beneficial effects of estrogen on reducing β -amyloid plaques (Levin-Allerhand et al 2002). Furthermore, a separate study found that the gene encoding transthyretin, which has been reported to scavenge β -amyloid peptides and reduce amyloid plaque formation was found to be increased in ovariectomised mice treated with 17β -estradiol when compared to placebo treated ovariectomized mice (Tang et al 2004). Other studies using amyloid precursor protein transgenic mice have reported no effect of ovariectomy or estrogen replacement on β -amyloid deposition in the hippocampus and neocortex (Green et al 2005; van Groen and Kadish 2005). However, the estrogen deficient APP23 mice (generated by cross-breeding the APP23 transgenic mouse (AD model) with the ArKO mouse) presented amyloid plaques formation at a younger age, accompanied by an increased β -amyloid peptide deposition and increased β -amyloid production (Yue et al 2005). Cell

cultures from the brains of these mice also showed a marked impairment in β -amyloid clearance and degradation (Yue et al 2005). In fact, Yue et al reported that female AD brain contained greatly reduced estrogen levels compared with those from age- and gender-matched normal control subjects; AD and control subjects had comparably low levels of serum estrogen. These results support the concept that locally produced estrogens in the brain play important roles in neuroprotection.

A more recent report noted differential effects of the two ER subtypes on the expression of apolipoprotein E as a risk factor for Alzheimer's disease, indicating that past differences may be due to differences in the ER subtype expression within the specific brain regions analyzed (Wang et al 2006). These *in vivo* studies certainly strengthen *in vitro* experiments demonstrating the strong neuroprotective properties of estrogen.

The ER α KO mouse has also been an efficient model to determine the predominant ER α involvement in estrogen regulation of satiation signaling, feeding effects (Geary et al 2001), and in estrogen regulation of gonadotropin signaling pathways and LH secretion from the pituitary (Couse et al 2003; Lindzey et al 1998). The ER α subtype was also shown to be the predominant receptor in estrogen mediated neuroprotection from ischemic and stroke induced insults, by comparing ER α KO to WT mice (Ardelt et al 2005; Dubal et al 2001). Significant morphological abnormalities have been found in the brains of ER β KO mice. There is a regional neuronal hypocellularity, with severe neuronal deficit in the somatosensory cortex, particularly in layers II, III, IV and V, and this is accompanied by an intense proliferation of astroglial cells in the limbic system but not in the cortex (Wang et al 2001). This phenotype is exacerbated with age (Wang et al 2001). Put together, these studies highlight the usefulness of all three ERKO models (ER α KO, ER β KO and ER $\alpha\beta$ KO) in determining the pathways involved in estrogen mediated neuroprotection.

In fact some studies have found that the use of ER α KO and ER β KO models has actually led to the discovery that neither ER subtype may be involved in estrogen regulated neuroprotective mechanisms, and rather the proposed third membrane bound estrogen receptor is responsible. Indeed the membrane bound or ER-X receptor is believed to be responsible for estrogen regulated activation of the mitogen-activated protein (MAP) kinase cascade (Singh et al 2000; Toran-Allerand 2000). Furthermore the ER-X receptor, which is believed to be situated within the caveolar-like microdomains of postnatal but not adult WT and ERKO neocortex and uterine plasma membranes, is re-expressed in the adult brain following ischemic stroke injury (Toran-Allerand et al 2002). Such studies represent great prospects in rapid acting neuroprotective actions of estrogens in the therapeutic setting.

MODULATING EFFECTS OF ESTROGENS ON BEHAVIOR

Estrogens have numerous effects on the brain throughout the lifespan beginning during gestation and continuing on into senescence. Estrogens participate in the sexual differentiation of the brain during early neonatal life and affect areas of the brain that are not primarily involved in reproduction, such as the basal forebrain cholinergic system, the hippocampus and cerebral cortex, the caudate putamen, midbrain raphe and brain stem locus coeruleus and the spinal cord. Both estrogen receptors, ER α and ER β , are expressed in high density in several brain regions associated with mood, cognition, and motor control (Shughrue, P.J., Lane, M.V. & Merchenthaler, I. (1997) Comparative distribution of estrogen receptor-a and -b mRNA in the rat central nervous system. *J Comp Neurol* 388, 507–525.). These systems are involved in a variety of estrogen actions on mood, cognition, pain sensitivity, and vulnerability to mental illnesses.

Learning and Memory

Estragens have been demonstrated to enhance some aspects of cognitive function in animal and human models. However, the demonstrated effects are often not large and inconsistent across studies (see review by Luine VN *J Neuroendocrinol.* 2008 Jun;20(6):866-72. Sex steroids and cognitive function). Conflicting results have also been reported in the estrogen deficient models.

ArKO mice both sexes performed significantly worse than WT controls in tests for short term memory by use of the Y-maze test, thus revealing an important role for estrogen in the memory process (Martin et al 2003). Similarly, male and female 3-month-old ER β KO mice show profound memory impairment in a hippocampus-mediated fear-conditioning paradigm.

Using the Morris Watermaze, WT males and ER α KO of both sexes, treated with estradiol benzoate (EB) or oil, exhibited decreased latencies across blocks of trials in the Morris Watermaze (which test the spatial learning ability of the rodents); whereas WT females treated with EB failed to learn this spatial discrimination task (*Horm Behav.* 1998 Oct;34(2):163-70. Sex differences in the activational effect of ER α on spatial learning. Fugger HN, Cunningham SG, Rissman EF, Foster TC). The Morris Watermaze performance of the female ArKO mice were similar to the WT counterparts (Boon et al 2005).

Aggressive Behavior

Estrogen has a facilitatory effect on male aggressive behavior which has been demonstrated clearly through analyses of the ArKO and ERKO. It has been reported that the ArKO males failed to display aggressive behavior against intruder mice (*J Endocrinol.* 2001 Feb;168(2):217-20. A loss of aggressive behaviour and its reinstatement by oestrogen in mice lacking the aromatase gene (Cyp19). Toda K, Saibara T, Okada T, Onishi S, Shizuta Y). This deficit could be restored if the mice received 17 β -estradiol replacement soon after birth, although the restored behavior display lasted a shorter period when compared to the WT. If the 17 β -estradiol replacement was delayed till 1 week of age, no aggression could be observed from the treated ArKO

(J Endocrinol. 2001 Feb;168(2):217-20. A loss of aggressive behaviour and its reinstatement by oestrogen in mice lacking the aromatase gene (Cyp19). Toda K, Saibara T, Okada T, Onishi S, Shizuta Y). These findings clearly illustrate an absolute requirement for estrogen during the neonatal stage of a male's life for the development of the potential for aggression observed in adulthood.

In line with the observations of ArKO, the male ER α KO rarely displayed aggressive behavior or male-typical offensive attacks (Proc Natl Acad Sci U S A. 1997 Feb 18;94(4):1476-81. Behavioral effects of estrogen receptor gene disruption in male mice. Ogawa S, Lubahn DB, Korach KS, Pfaff DW). Daily injection of testosterone propionate could not induce aggressive behavior in castrated male ER α KO mice but restored aggressiveness in castrated WT (Endocrinology. 1998 Dec;139(12):5058-69. Modifications of testosterone-dependent behaviors by estrogen receptor-alpha gene disruption in male mice. Ogawa S, Washburn TF, Taylor J, Lubahn DB, Korach KS, Pfaff DW).

By contrast, male ER β KO mice are more aggressive than WT males; injections of estradiol benzoate further heightened aggressive behavior in the former (Eur J Neurosci. 2006 Apr;23(7):1860-8. Estrogen receptor-beta gene disruption potentiates estrogen-inducible aggression but not sexual behaviour in male mice. Nomura M, Andersson S, Korach KS, Gustafsson JA, Pfaff DW, Ogawa S). However, age has an attenuating effect on this aggressive phenotype which could be explained by the declining serum levels of testosterone. Therefore, these findings demonstrated the male aggression induced by estrogen through ER α -mediated brain mechanisms may be attenuated by ER β activation.

Anxiety

Anxiety has often been associated with low levels of estradiol in postmenopausal women. Consistent with these observations in humans, increased anxiety was observed principally in ER β KO (but not ER α KO females) with a concomitant increase of 5-hydroxytryptamine 1a receptor expression in medial amygdala (Proc Natl Acad Sci U S A. 2001 Oct 9;98(21):12278-82. Increased anxiety and synaptic plasticity in

estrogen receptor beta -deficient mice. Krezel W, Dupont S, Krust A, Chambon P, Chapman PF) as well as significantly lower serotonin (5-HT) content than WT littermates in several brain regions including: the bed nucleus of the stria terminalis, preoptic area, and hippocampus. (Physiol Behav. 2005 Jan 31;84(1):157-63. Lack of functional estrogen receptor beta influences anxiety behavior and serotonin content in female mice. Imwalle DB, Gustafsson JA, Rissman EF). Supporting these data was the report that selective ER β agonist diarylpropionitrile (DPN) treated rats showed significantly decreased anxiety-related behavior in the elevated plus maze or open field whereas the ER α -selective agonist propyl-pyrazole-triol had no such effects. The effects of DPN could be blocked by Tamoxifen (Endocrinology. 2005 Feb;146(2):797-807. Novel actions of estrogen receptor-beta on anxiety-related behaviors.Lund TD, Rovis T, Chung WC, Handa RJ) On the other hand, female ArKO (i.e. the complete estrogen-deficient model) mice neither show increase anxiety in the Open Field Test when compared to the WT controls nor changes in the levels of serotonin or catecholamines in the hypothalamus, prefrontal cortex or striatum. Nonetheless, an increased serotonergic activity was observed in the ArKO hippocampus (Eur J Neurosci. 2004 Jul;20(1):217-28. Oestrogen-deficient female aromatase knockout (ArKO) mice exhibit depressive-like symptomatology. Dalla C, Antoniou K, Papadopoulou-Daifoti Z, Balthazart J, Bakker J).

Estrogen has been shown to modulate serotonin levels (Alves et al 2000; Gundlah et al 2005). However, differential effects of estrogens depend on ER subtype expression within the specific brain region. For example, using ERKO animals, it was demonstrated that ER α mediated estrogen induced progesterone and possible tryptophan hydroxylase-1 (TPH; serotonin synthesizing enzyme) expression in the hippocampus (Alves et al 2000) whereas ER β is responsible for mediating estrogen induced TPH expression in the mouse dorsal raphe nucleus (DRN; the largest serotonergic nucleus) (Gundlah et al 2005). The latter study correlates with previous reports which show that estrogen and progesterone modulation of serotonergic function, is regulated via ER β , as no differences in progesterone receptor, ER α and TPH immunoreactive co-localization expression was seen in WT compared to ER α KO mice (Alves et al 2000).

Depression

In rodents, decreased active behaviors such as struggling or swimming and increased passive behavior such as floating during a forced swim test situation are indicator of depressive-like symptoms. Indeed, female adult ArKO mice demonstrated such depressive-like behavior which were accompanied by a concomitant increase in serotonergic activity within the hippocampus of ArKO female compared to WT controls (Dalla et al 2004). These phenotypes could not be ameliorated by estradiol treatment, suggesting that they may be results of the absence of estrogens during development. In contrast, male ArKO mice did not such depressive-like symptomology (Dalla et al 2005). This seemed to mirror the report that approximately twice as many women suffers from depression as men, across all age groups (reviewed by Gorman JM 2006) at least in the United States.

The anti-depressive effects of estrogens were demonstrated by administering SERMS (selective estrogen receptor modulators) to ovariectomised rodents. Administration of ER β -selective SERMs to the hippocampus, but not the ventral tegmental area, decreased anxiety and depressive behavior (Walf AA, Frye CA. A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. *Neuropsychopharmacology*. 2006 Jun;31(6):1097-111). When the ER β was disrupted, as in the ER β KO, the anti-depressive effects of 17 β -estradiol was not observed (Beatriz A. Rocha . Rebecca Fleischer .James M. Schaeffer . Susan P. Rohrer . Gerry J. Hickey 17 β -Estradiol-induced antidepressant-like effect in the Forced Swim Test is absent in estrogen receptor- β knockout (BERKO) mice.

Prepulse inhibition

Prepulse inhibition is a model of sensorimotor gating mechanisms necessary for normal sensory information processing in the brain (Braff, D.L. & Geyer, M.A. (1990) Sensorimotor gating and schizophrenia. Human and animal

model studies. Arch General Psychiatry 47, 181–188). Deficiencies in prepulse inhibition have been found in patients with schizophrenia (Braff, D.L., Swerdlow, N.R. & Geyer, M.A. (1995) Gating and habituation deficits in the schizophrenia disorders. Clin Neurosci 3, 131–139. Braff et al. 1995), Tourette's syndrome (Castellanos, F.X., Fine, E.J., Kaysen, D., Marsh, W.L., Rapoport, J.L. & Hallett, M. (1996) Sensorimotor gating in boys with Tourette's syndrome and ADHD: preliminary results. Biol Psychiatry 39, 33–41.), Obsessive Compulsive Behavior (Hoenig K, Hochrein A, Quednow BB, Maier W, Wagner M. Impaired prepulse inhibition of acoustic startle in obsessive-compulsive disorder. Biol Psychiatry. 2005 May 15;57(10):1153-8.) Autism (Biol Psychiatry. 2007 Feb 15;61(4):482-6. Sensorimotor gating deficits in adults with autism. Perry W, Minassian A, Lopez B, Maron L, Lincoln A).

Gender in prepulse inhibition have been described with women showing less inhibition than men especially when weaker prepulse was used (Swerdlow, N.R., Auerbach, P., Monroe, S.M., Hartston, H., Geyer, M.A. & Braff, D.L. (1993) Men are more inhibited than women by weak prepulses. Biol Psychiatry 34, 253–260.). However, no such sexual dimorphism was observed in rats in the same study. By contrast, gender differences were observed in the ArKO mice - male, but not female ArKO mice, present an age-related reduction of prepulse inhibition (Prepulse inhibition of acoustic startle in aromatase knock-out mice: effects of age and gender M. van den Buuse, E. R. Simpson and M. E. E. Jones. Genes, Brain and Behavior (2003) 2: 93–102) It is, however, not known which estrogen receptor mediated the modulation effects of estrogen on the sensory gating mechanism.

Running Wheel Activity

Estrogens are known to increase running wheel activity of rodents primarily by acting on the ER α and ER β positive medial preoptic area (mPOA) (Fahrbach et al 1985, King 1979, Morgan and Pfaff 2001 and Roy and Wade 1975). It has been shown that both gonadectomised female and male ER α KO mice had similar level of running wheel activity as the corresponding gonadectomised WT mice in placebo control groups. Estrogen treatment increased the running wheel activity of both WT and ER β KO females and males but not ER α KO mice (Endocrinology. 2003 Jan;144(1):230-9. Estrogen increases

locomotor activity in mice through estrogen receptor alpha: specificity for the type of activity. Ogawa S, Chan J, Gustafsson JA, Korach KS, Pfaff DW), thus demonstrating that the up-regulating effects of estrogens on running wheel activity is mediated via ER α .

Recently, we reported that adult male ArKO mice displayed a significantly increase running wheel activity but a decrease in normal ambulatory activity when compared to WT (Hill *et al* 2007). This specific behavior of excessive running wheel activity was returned to WT levels upon estrogen replacement (Hill *et al* 2007). In contrast, in female ArKO mice displayed a non-significant decrease in running wheel activity compared to WT.

Grooming

Excessive grooming behavior has commonly been referred to as a form of compulsive related behavior in both animals and humans (Ferris *et al* 2001, Greer and Capecchi 2002, Nordstrom and Burton 2002 and Rapoport 1991). We reported that the male ArKO mice displayed excessive grooming behavior that are at a significantly higher level than those of their WT littermates (Hill *et al* 2007). This excessive grooming activity of the male ArKO was sufficiently restored to WT levels following three weeks of 17 β -estradiol replacement (Hill *et al* 2007), thus indicating that in adult males, induced grooming activity via a water mist spray may be regulated by levels of estrogen. No differences in grooming behavior were observed in female ArKO compared to WT, indicating that estrogen actions on this compulsive-related behavior are specific to males only (Hill *et al* 2007).

The medial preoptic area previously shown to regulate both grooming (Lumley *et al* 2001) and running wheel behaviors (Fahrbach *et al* 1985 and King 1979). Indeed the COMT catechol-O-methyltransferase (COMT) in the male ArKO hypothalamus was significant lowered but not in the frontal cortex. COMT is involved in the metabolism of dopamine, hence a possible consequence would be an increased dopamine level in the hypothalamus leading to development of excessive

grooming and running wheel activities in the male ArKO. By contrast, no such behavior phenotypes were observed in the female ArKO mice which is paralleled by the normal presentation of hypothalamic COMT levels.

As estrogen is required during neuronal development, we cannot rule out the possibility that the OCD related phenotype presented in the male ArKO mice is a result of estrogen deficiency during development. Nonetheless, just 3 weeks of 17 β -estradiol replacement did restore the COMT levels in the ArKO hypothalamus with concomitant amelioration of the compulsive behavior (Hill *et al* 2007). Thus, decreased estrogen levels correlate with a specific decrease in hypothalamic COMT expression and development of compulsive behavior (i.e. grooming and wheel-running in male mice).

Sexual Behavior

Male ER α KO mice showed reduced levels of intromission and ejaculation (Ogawa *et al* 1997),. Although their mounting behavior were unaffected (Ogawa *et al* 1997), male ER α KO mice did not exhibit the same social preferences for female mice as do their WT littermates (Rissman *et al* 1999). In contrast, male ER β KO mice displayed all components of sexual behavior, with no apparent effect of genotype (Ogawa *et al* 1999). However, the double ER $\alpha\beta$ KO did not show any components of sexual behavior, including mounting behavior (Ogawa *et al* 2000) which was similar to the male ArKO sexual behavior phenotype reported (Robertson KM, Simpson ER, Lacham-Kaplan O, Jones ME. Characterization of the fertility of male aromatase knockout mice. *J Androl.* 2001 Sep-Oct;22(5):825-30). A role for ER β has been suggested in the defeminization of sexual behavior, castrated male ER β KO mice showed significantly higher levels of female receptivity as compared with WT littermates after treated with female priming hormones. (Proc Natl Acad Sci U S A. 2005 Mar 22;102(12):4608-12. A previously uncharacterized role for estrogen receptor beta: defeminization of male brain and behavior. Kudwa AE, Bodo C, Gustafsson JA, Rissman EF).

Female ER α KO mice were infertile and did not appear to engage in reproductive behavior. They did not display any sexual behavioral characteristics such as immobilization or vertebral dorsiflexion even after treatment with strong cutaneous stimuli which usually promote lordosis behavior (see review Ogawa et al 1996). This was further confirmed by the work of Rissman and colleagues who found by use of the ER α KO model that ER α is indeed required for sexual receptivity but not essential for female attractivity (Rissman et al 1997). Similarly, sexual receptivity was severely impaired in ER β KO (J Neuroendocrinol. 2003 Oct;15(10):978-83. Double oestrogen receptor alpha and beta knockout mice reveal differences in neural oestrogen-mediated progesterin receptor induction and female sexual behaviour.Kudwa AE, Rissman EF.). In contrast, female ER β KO females exhibited lordosis behavior equal to that of WT females (J Neuroendocrinol. 2003 Oct;15(10):978-83. Double oestrogen receptor alpha and beta knockout mice reveal differences in neural oestrogen-mediated progesterin receptor induction and female sexual behaviour.Kudwa AE, Rissman EF.). In sum, estrogens are required for the development of female sexual behavior.

One study has shown that ArKO females were not receptive even after priming with estradiol and progesterone that successfully induced receptivity in WT females (J Neurosci. 2002 Oct 15;22(20):9104-12. The aromatase knock-out mouse provides new evidence that estradiol is required during development in the female for the expression of sociosexual behaviors in adulthood.Bakker J, Honda S, Harada N, Balthazart J). However, another study reported that when ArKO females were raised on chow that is low in phytoestrogen content, they were capable of displaying levels of lordosis equivalent to WT littermates (Behav Neurosci. 2007 Apr;121(2):356-61. Dietary phytoestrogens dampen female sexual behavior in mice with a disrupted aromatase enzyme gene.Kudwa AE, Boon WC, Simpson ER, Handa RJ, Rissman EF). The receptivity of ArKO was significantly decreased when raised on standard laboratory chow (which contained high phytoestrogen content) although the receptivity of WT was not affected by consumption levels of phytoestrogen. It is not possible to compare the 2 studies as the phytoestrogen content of the first study was not stated. Nonetheless, the present findings showed that the phytoestrogen can affect the behavior of animals. Phytoestrogens can have agonistic or antagonistic

actions on ER (Patisaul, H. B. (2005). Phytoestrogen action in the adult and developing brain. *Journal of Neuroendocrinology*, 17, 57–64.), which may be modulated by the presence of endogenous estrogens (Breithofer, A., Graumann, K., Scicchitano, M. S., Karathanasis, S. K., Butt, T. R., & Jungbauer, A. (1998). Regulation of human estrogen receptor by phytoestrogens in yeast and human cells. *Journal of Steroid Biochemistry and Molecular Biology*, 67(5–6), 421–429; Kuiper, G. G., Lemmen, J. G., Carlsson, B., Corton, J. C., Safe, S. H., van der Saag, P. T., et al. (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*, 139, 4252–4263.).



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