Estrogen signaling as a therapeutic target in neurodevelopmental disorders

Amanda Crider and Anilkumar Pillai

Department of Psychiatry and Health Behavior, Medical College of Georgia, Augusta University, Augusta, GA 30912.
Running Title: Estrogen signaling, ASD and schizophrenia

Address for Correspondence:
Anilkumar Pillai, Ph.D.
CA-3143
1462 Laney Walker Blvd
Augusta University
Augusta, GA 30912
Phone – (706) 446-0325
apillai@augusta.edu

Word count:
Abstract: 142
Manuscript: 5878
Number of Tables: 1
Number of Figures: 1

Abbreviations: ADHD, Attention Deficit Hyperactivity Disorder; ASD, autism spectrum disorder; BDNF, brain derived neurotrophic factor; DHEA, didehydroepiandrosterone; ER, estrogen receptor; GPR30, g-protein coupled receptor 30; GPER, g-protein coupled estrogen receptor; FXS, Fragile X syndrome; IFN-g, interferon-g; IL, interleukin; MPP, 1,3-Bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; PPT, 4,4',4''-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol; PSD-95, postsynaptic density protein 95; SERM, synthetic estrogen receptor modulator.
Estrogens, the primary female sex hormones, were originally characterized through their important role in sexual maturation and reproduction. However, recent studies have shown that estrogens play critical roles in a number of brain functions including cognition, learning and memory, neurodevelopment and adult neuroplasticity. A number of studies from both clinical as well as preclinical research suggest a protective role of estrogen in neurodevelopmental disorders including autism spectrum disorder (ASD) and schizophrenia. Alterations in the levels of estrogen receptors (ERs) have been found in subjects with ASD or schizophrenia, and adjunctive estrogen therapy has been shown to be effective in enhancing the treatment of schizophrenia. This review summarizes the findings on the role of estrogen in the pathophysiology of neurodevelopmental disorders with a focus on ASD and schizophrenia. We also discuss the potential of estrogen as a therapeutic target in the above disorders.
INTRODUCTION

Estrogen was first isolated and characterized in 1929 by Tadeus Reichstein, Adolf Butenandt, and Edward Adelbert Doisy (Tata, 2005). The field of endocrinology progressed rapidly and in the 1950s, it was discovered that there was major interaction between the endocrine and central nervous systems, coordinating activities of metabolic and developmental processes in response to the environment (Tata, 2005). In the 1960s, it was found that hormones affect transcription and translation of mRNA and protein. Estrogen was specifically implicated in transcriptional activation in the 1970s and 1980s by Pierre Chambon and Bert O’Malley (Tata, 2005). Along with the discovery of hormonal regulation of genes came the knowledge of hormone receptors.

Estrogen is produced centrally by the brain and peripherally by the ovaries and other tissues (Nelson and Bulun, 2001; Gruber et al., 2002). Estrogen plays different roles in each tissue and is involved in puberty, fertility, estrus cycle, brain development, and neuroprotection (Cui et al., 2013). Estrogen is produced from testosterone in the ovaries, corpus luteum, and placenta in premenopausal women (Cui et al., 2013). It can also be produced in the liver, heart, skin, adipose tissue, and brain (Nelson and Bulun, 2001; Gruber et al., 2002; Cui et al., 2013). Estradiol is the bioactive form of estrogen, but other forms of estrogen are produced by females (estrone and estriol). Aromatase catalyzes the final step in estradiol synthesis and is widely expressed in many tissues, but many tissue-specific forms exist (Simpson et al., 1997). Similar to peripheral synthesis, brain estrogen synthesis can occur from testosterone, but it can also be produced de novo (Cui et al., 2013). In the brain, estrogen is produced in many regions including the hippocampus, cortex, cerebellum, hypothalamus, and amygdala (Cui et al., 2013). Neurons are the primary site of estrogen synthesis in the brain, but it can also be produced by astrocytes,
but not microglia or oligodendrocytes (Azcoitia et al., 2011). Estrogen synthesis and aromatase expression are involved in neural development, synaptic plasticity, and cell survival (Azcoitia et al., 2011). The specificity of aromatase expression and therefore estradiol production in certain cell types and brain regions suggests that local production of estradiol is important for specific brain processes (Cui et al., 2013).

A number of recent studies have shown that estrogen plays a critical role in the pathophysiology of neurodevelopmental disorders. ASD is a neurodevelopmental disorder that affects 1 in 68 children in America and approximately 1% of the worldwide population. Individuals with ASD exhibit a wide range of symptoms including anxiety, depression, repetitive and obsessive behaviors, language and communication deficits, and social deficits. These symptoms occur over a spectrum of severity and can appear in a heterogeneous fashion in patients with the disorder. ASD appears to be gender specific, affecting boys 5x more often than girls, which rises to 10x more often in Asperger’s syndrome, a high-functioning form of ASD. The increased risk for males to develop ASD suggests a potential role of sex hormones in the pathophysiology of this disorder. Schizophrenia is another neuropsychiatric disorder with neurodevelopmental origin (Hoftman et al., 2016; Lewis and Levitt, 2002; Weinberger, 1995). Subjects with schizophrenia typically present with three types of symptoms: positive (in addition to normal behavior), negative (deficits), and cognitive (Kulkarni et al., 2012). These symptom sets include hallucinations, delusions, catatonic behavior, disorganized speech, depression, flat affect, and social deficits in early adulthood (Association, 2013; NAMI, 2013; McGrath et al., 2008). Schizophrenia can also present earlier in life, which is denoted as childhood-onset schizophrenia. This disorder affects about 1% of individuals during their lifespan (McGrath et
al., 2008). A number of comorbid conditions including substance abuse, reduced lifespan, and suicide are often seen in subjects with schizophrenia (Kulkarni et al., 2012).

Many factors appear to be significant in the development of ASD and schizophrenia. A number of factors, both genetic and environmental have been implicated in the pathogenesis of these two disorders. Understanding the pathophysiology is very critical for the development of novel therapeutics and long-term treatment management of ASD and schizophrenia. This article will provide an overview of the recent progress of our understanding in the role of estrogen in neurodevelopmental disorders with a focus on ASD and schizophrenia.

**ESTROGEN SIGNALING AND BRAIN FUNCTION**

There are three known receptors for estrogen, estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), and g-protein coupled receptor 30 (GPR30), which is also known as g-protein coupled ER1 (GPER). ERα (then known as ER) was discovered to specifically bind estrogen in 1986 (Green et al., 2006; Greene et al. 1986). In 1995, an additional estrogen receptor was cloned and named ERβ (Kuiper et al., 1996), explaining why estrogen effects were still seen in ERα knockout mice. A third receptor with homology to g-protein coupled receptors is a membrane-bound receptor that binds estrogen and possibly other ligands (Maggiolini and Picard, 2010).

ERα is thought to be responsible for modulating neurobiological reproductive systems such as sexual characteristics and puberty (Behl, 2002; De Fossé, 2004). ERβ is important in modulating non-reproductive neurobiological systems that are involved in anxiety, locomotion, fear, memory, and learning (Krezel et al., 2001). ERβ is the principal estrogen receptor expressed in cortex, hippocampus, and cerebellum (Bodo and Rissman, 2006). A number of
Cofactors have been shown to regulate the expression of ERα and ERβ at the transcriptional level. Some of these include: steroid receptor coactivator-1 (SRC-1), transcriptional mediators/intermediary factor 2 (TIF2), amplified in breast 1 (AIB1), CREB binding protein (CBP), and CBP-associated factot (CAF), which are coactivators, as well as silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor corepressor (nCOR) which are corepressors (Bodo and Rissman, 2006). Estrogen receptors can also regulate the production of proteins through genetic loci called estrogen response elements (EREs). These regions allow direct binding of estrogen receptors to the DNA at specific loci, which can alter gene expression. GPER appears to mediate many of the rapid, non-genomic actions of estrogen and typically involve regulation of membrane bound and cytoplasmic regulatory proteins. (Huang et al., 2016). GPER is expressed in the plasma membrane (Filardo and Thomas, 2005; Filardo and Thomas, 2012; Cheng et al., 2011), endoplasmic reticulum and trans-Golgi network (Revankar et al., 2005). Moreover, its plasma membrane localization is stabilized by association with scaffolding proteins containing PDZ (Dlg homologous region or (glycine-leucine-glycine-phenylalanine domain) binding domains, such as post synaptic density protein 95 and synapse associated protein 97, as well as with other g-protein coupled receptors (GPCRs) (Akama et al., 2013; Waters et al., 2015; Broselid et al., 2014). In the brain, GPER is widely expressed in hippocampus, cortex and hypothalamus (Prossnitz and Arterburn, 2015).

**Neuroprotective effects of estrogen**

Estrogen is largely thought to be neuroprotective. The bioactive form of estrogen known as 17β-estradiol (E2) has been implicated in neuroprotection, cognitive functioning, and synaptic plasticity (Yang et al., 2010). On the other hand, chronic estrogen deprivation has also been shown to increase risk of neurological disorders such as stroke, Alzheimer’s disease, Parkinson’s
disease (Scott et al., 2012). The neuroprotective effects of estrogen occur through genomic and non-genomic signaling, antioxidant functions, and the maintenance of neuronal adenosine triphosphate (ATP) through estrogen receptors (Brann et al., 2007). Estrogens can upregulate antiapoptotic genes and downregulate proapoptotic genes through diffusion of estradiol through the cell membrane and translocation and binding of estrogen receptors to genes in the nucleus, promoting or inhibiting transcription (Scott et al., 2012; Choi et al., 2004; Dubal et al., 1999). This regulation seems to be largely dependent on ERα and occurs on the scale of hours (Scott et al., 2012; Dubal et al., 2001). Estrogen also exerts neuroprotective function through nongenomic signaling and localize in the plasma membrane of cortical and hippocampal neurons (Scott et al., 2012; Dubal et al., 2001). These receptors are thought to be involved in more rapid estrogen signaling including regulation of kinases, calcium signaling, and other pathways that occur on the scale of minutes to hours (Scott et al., 2012). Estradiol also protects the brain through its ability to increase cerebral blood flow, facilitation of glucose metabolism (Brinton, 2008; Yao et al., 2012), and enhancement of electron transport chain activity to supply energy to neurons (Choi et al., 2004; Yao et al. 2010). It is important to note that there appear to be “critical periods” or periods of effectiveness for estrogen neuroprotection (Choi et al., 2004; Sherwin, 2009). The critical period hypothesis states that estrogen therapy must be given within a certain period of time following menopause (Sherwin, 2009). This theory follows hand in hand with the healthy cell bias theory of estrogen which states that estrogen therapy is only effective when applied to healthy neurons (Brinton, 2005). Animal studies have shown that estrogen can exert neuroprotective effects against ischemic damage if it is replaced immediately after injury, but not at 10 weeks post-ovariectomy (Scott et al., 2012). Also, long-term estrogen deprivation causes tissue-specific reduction in ERα levels in hippocampus, altering its sensitivity to estrogen,
explaining the need for immediate estrogen replacement for neuroprotection to be effective (Scott et al., 2012). A similar reduction in ERα has been shown to occur naturally during the aging process, altering hippocampal sensitivity to estrogen (Scott et al., 2012).

Estrogen is known to promote the synthesis of neurotrophins and protects the brain against inflammation and stress. Accumulating evidence suggest that estrogen regulates the expression of brain derived neurotrophic factor (BDNF), a key molecule involved in neuronal survival, differentiation, and synaptic plasticity (Blurton-Jones et al., 2003, Numakawa et al., 2010, Solum and Handa, 2002 and Zhou et al., 2005). ER mediated transcription has been shown to be potentiated by full length TrkB, the receptor for BDNF (Wong et al., 2013). Estrogen has also been shown to regulate BDNF mRNA and protein expression (Solum and Handa, 2002). In addition, estrogen treatment has been shown to restore the reduced expression of BDNF mRNA in the midbrain area of ovariectomized mice (Yi et al., 2016). Estradiol has also been shown to exert anti-inflammatory activity on activated macrophages and microglia (Dodel et al., 1999; Drew and Chavis, 2000; Bruce-Keller et al., 2000, Vegeto et al., 2001, Vegeto et al., 2003 and Vegeto et al., 2004). Estrogen is known to reduce endoplasmic reticulum stress, an inducer of inflammation, through a synovilin 1-dependent mechanism (Kooptiwut et al., 2014; Rajapaksa et al., 2014). It has been shown that ovariectomy is associated with changes in the peripheral immune response as evidenced by increased levels of inflammatory markers such as TNF-α, IL-1β, macrophage inflammatory protein-1, and macrophage colony-stimulating factor (Benedusi et al., 2012 and Cenci et al., 2000). The NF-kB family of transcription factors regulates many genes that play important roles in the function of the innate and adaptive immune systems. Moreover, estrogen-induced activation of the ER results in a reduction in the levels of nuclear DNA-binding activity of NF-kB (Kalaitzidis and Gilmore, 2005), which in turn regulates the expression of
inflammatory genes (O’Neill and Kaltschmidt, 1997). Interestingly, treatment of ovariectomized mice with 17α-ethinylestradiol has been shown to suppress NF-KB-induced inflammatory genes (Evans et al., 2001). Moreover, treatment with estrogen modulators has been shown to reduce levels of NFkB and the activation of t-cells (Bebo et al., 2009; Lee et al., 2008). Studies conducted using ERα knockou... 

**Estrogen in neurodevelopment and plasticity**

Estradiol is extremely important in brain development. The fetal brain is exposed to maternal estradiol, and that which is locally produced by the fetus (McCarthy, 2008). The developing brain shows high expression levels of estrogen receptors, which regulate gene expression and signal transduction (McCarthy, 2008). This expression is somewhat sex-specific and varies over time as brain development progresses (McCarthy, 2008; Yokosuka et al., 1997).

Estrogen’s effects on learning and memory occur through rapid, non-genomic signaling pathways. Acute treatment with 17β-estradiol rapidly increased the activation of extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K) (Fernandez et al., 2008; Fan et al., 2010). Estradiol is necessary for hippocampal, frontal cortex, cerebellar, and basal forebrain-based learning and spatial memory in rats (Luine et al., 1998; Shang et al., 2010; Andreeescu et al., 2007). Also, ovariectomized rats have shown impaired performance in working memory and spatial navigation tasks, and acute activation of estrogen signaling using 17β-estradiol or other potent estrogens, including synthetic estrogen receptor modulators (SERMs) could reverse the above deficits (Daniel et al., 1997; Bimonte and Denenberg, 1999; Gibbs,
In male mice, administration of 17β-estradiol has been shown to improve performance in inhibitory and water maze learning tasks (Frye et al., 2005). Estradiol can alter epigenetic processes within minutes to improve consolidation of hippocampal memories (Bi et al., 2000, Fernandez et al., 2008). In vitro studies have shown that estradiol activation of some of the same epigenetic pathways also promotes dendritic spine remodeling, which suggests a link between spinogenesis and memory consolidation (Hasegawa et al., 2015; Kramár et al., 2009). The knowledge that estradiol can be produced locally in the hippocampus further suggests that rapid estrogen signaling contributes to memory formation (Kretz et al., 2004; Prange-Kiel et al., 2006).

Aromatase, encoded by the cyp19 gene, is the rate-limiting step in the biosynthesis of estrogens, and is widely distributed in different regions of the brain including hypothalamus, hippocampus, and cortex (Rune and Frotscher, 2005; Yague et al., 2006; Boon et al., 2010; Azcoitia et al., 2011; Saldanha et al., 2011). It is expressed in both neurons and glial cells (Kretz et al., 2004; Yague et al., 2008). Mice deficient in aromatase show impairments in spatial reference memory (Martín et al., 2003). Interestingly, treatment with estradiol benzoate and dihydrotestosterone propionate has been shown to restore social recognition abilities in castrated aromatase KO mice (Pierman et al., 2008) further suggesting the important role of estrogen in behavior.

Studies focused on specific estrogen receptor isoforms have further established the role of estrogen signaling in brain functions. Accumulating literature suggest that ERβ primarily regulates non-reproductive components of estrogen signaling in the brain (Weiser et al., 2008; Bodo and Rissman, 2006). ERα knockout (KO) mice show severe deficits in reproduction and some alterations in learning (Rissman et al., 1999). ERβ KO mice show normal sexual behavior...
and only slight reproductive deficits, but severe memory and learning deficits (Rissman et al., 2002). Modulation of ERβ enhances spatial memory while ERα facilitates sexual behavior (Rhodes et al., 2006). It has been shown that ERα KO, but not ERβ KO, mice showed an improvement in cognitive function following treatment with 17β-estradiol further indicating a critical role for ERβ in mediating rapid estrogenic regulation of cognitive function (Liu et al., 2008). ERβ is also important for motor learning by potentiating cerebellar plasticity and synaptogenesis (Khan et al., 2014).

A key mechanism involved in the effects of estrogen on improving cognitive function is through the regulation of synaptic structure and function (Srivastava et al., 2013). Seminal studies by McEwan and colleagues have shown that 17β-estradiol treatment could restore ovariectomy-induced loss of dendritic spine density in the hippocampal pyramidal neurons (Gould et al., 1990; Woolley et al., 1990). Additional studies have demonstrated that 17β-estradiol increases spine number on cortical neurons in the prefrontal cortex of ovariectomized rhesus monkeys (Hao et al., 2007; Dumitriu et al., 2010). The above findings from in vivo studies on the effects of 17β-estradiol on spine density are further supported by in vitro studies. It has been shown that the decrease in synapse number in response to GABA(A) receptor blockade by bicuculline was restored by estradiol treatment in hippocampal neurons (Zhou et al., 2007). Similarly, aromatase inhibition using androstatrienedione has been shown to reduce dendritic spine density in cortical pyramidal neurons further suggesting an important role of estrogen in modulating spine density (Srivastava et al., 2008). In addition to estradiol, ERβ-selective agonist (WAY-200070, 7-bromo-2-(4-hydroxyphenyl)-1,3-benzoazol-5-ol) has also shown to increase the number of spines in cortical neurons (Srivastava et al., 2010). A recent study has reported that estradiol-induced spine changes in the dorsal hippocampus and medial prefrontal cortex are
mediated through ERK and mTOR signaling mechanisms in ovariectomized mice (Tuscher et al., 2016). Together, these findings suggest that estrogen signaling plays a critical role in synaptic function and plasticity.

ESTROGEN AND ASD

Many studies over the years have shown that increased testosterone exposure during pregnancy (Xu et al., 2015; Whitehouse et al., 2010; Auyeung et al., 2009; Auyeung et al., 2012; Tordjman et al., 1997; Palomba et al., 2012), decreased aromatase expression (Chakrabarti et al., 2009; Crider et al., 2014, Pfaff et al., 2011), and reduced estrogen or estrogen receptor expression (Crider et al., 2014; Chakrabarti et al., 2009) are significantly correlated with the development of ASD. Testosterone’s effects on behavior and the brain have been well characterized in human and animal studies. Increased prenatal testosterone exposure appears to be significantly more correlated than postnatal exposure with development of ASD. Increased testosterone during prenatal periods can result in social anxiety (Pfaff et al., 2011), reduced empathy and social development (Knickmeyer et al., 2006), and language development (Whitehouse et al., 2010). ASD and cognitive dysfunction have been correlated with precocious puberty, which is also caused by increased testosterone load (Tordjman et al., 1997).

The other side of the coin of increased testosterone is reduced estrogen signaling. In rodents, sex differences in the brain are largely driven by estradiol, which is produced locally by conversion of testosterone by aromatase (Wu et al., 2009). It follows that estradiol is also very important for proper brain development and cognition. Unlike testosterone, the effects of estrogen seem to be more profound postnatally. Reduction in estrogen signaling in adult mice through blocking aromatase or estrogen receptors has been shown to increase susceptibility to cognitive impairments due to repeated stress in rats (Wei et al., 2014). Adult females with low...
estrogen have also been shown to have impaired extinction and retrieval in fear extinction paradigms in rodents and humans (Milad et al., 2009; Milad et al., 2010). Reduced estrogen receptor expression in ASD has only been established very recently (Amin et al., 2005; Ostlund et al., 2003; Österlund et al., 2001; Chakrabarti et al., 2009; Crider et al., 2014). ERβ gene was found significantly associated with autism traits as measured by the Autism Spectrum Quotient and the Empathy Quotient in ASD subjects (Chakrabarti et al., 2009). In a recent study using postmortem brain samples from ASD and control subjects, we found that ERβ mRNA and protein levels are lower in the middle frontal gyrus of ASD subjects as compared to age- and gender-matched controls (Crider et al., 2014). In addition, significant reductions in aromatase (CYP19A1) mRNA and protein levels were observed in ASD subjects. CYP19A1 is enriched at synapses and localizes to presynaptic structures in neurons (Srivastava et al., 2010), suggesting that brain-synthesized estrogen plays an important role in neuronal function (Balthazart and Ball, 2006). The reductions in CYP19A1 could lead to impaired conversion of testosterone to estradiol resulting in increased levels of testosterone as observed in ASD subjects (Baron-Cohen et al., 2011). It is known that estrogen-ER complex recruits a variety of co-regulators that result in the activation or repression of target genes by modifying chromatin structure (Behl, 2002). We observed significant decreases in ER co-activators such as SRC-1, CBP and P/CAF mRNA levels in ASD subjects relative to controls (Crider et al., 2014). Together, these studies suggest that a coordinated regulation of ER and associated molecules plays an important role in ER signaling in the brain, and that this network may be impaired in subjects with ASD.

ESTROGEN AND SCHIZOPHREНИЯ

Schizophrenia is another major neurodevelopmental disorder where gender plays a very important role in the pathophysiology. Men with schizophrenia show a higher incidence, earlier
onset, and different symptomatology than women (Jablensky et al., 2000; Kulkarni et al., 2013). Women are not only less likely to develop the disorder, they are less likely to experience negative symptoms, substance abuse, and depression than men with schizophrenia (Conus et al., 2007). Men also show a higher incidence of conduct disorders, aggression, antisocial personality traits, higher levels of psychopathology, lower levels of treatment adherence, and lower levels of response to antipsychotics (Cotton et al., 2009; Morgan et al., 2008).

Along with the gender specificity of the disorder, there is evidence suggesting that estrogen levels correlate with the symptoms of schizophrenia. Several clinical studies have reported correlation between low plasma estrogen levels and an increase in the risk for schizophrenic symptoms in women (Mahé and Dumaine, 2001). An inverse correlation between the plasma estrogen levels across the menstrual cycle and psychopathological symptoms has been found in women with schizophrenia (Riecher-Rössler et al., 1994). Moreover, low rates of relapse have been observed in women with schizophrenia during pregnancy, when plasma estrogen levels are high (Chang and Renshaw, 1986 and Kendell et al., 1987). Additional studies have shown that serum levels of estradiol are associated with heightened well-being and improved cognition in healthy (Hampson, 1990) as well as schizophrenia subjects (Hampson, 1990; Akhondzadeh et al., 2003; Ko et al., 2006; Hoff et al., 2001). Two independent studies showed positive correlation between peripheral estrogen and cognitive performance in women with schizophrenia (Hoff et al., 2001; Ko et al., 2006). Another study using functional magnetic resonance imaging (fMRI) showed a significant positive correlation between sex steroid levels and brain activity in both women with schizophrenia and healthy men (Mendrek et al., 2011). Evidence also suggest estrogen may be the protective factor for women that reduce symptom severity and susceptibility (Kulkarni et al., 2012). For women who suffer from schizophrenia,
symptoms have been shown to be increased during the early follicular phase of the menstrual cycle, a phase where estrogen is particularly low (Bergemann et al., 2007; Rubin et al., 2010). In addition, the later onset of schizophrenia in women has been attributed to the protective effects of estrogen (Kulkarni et al., 2012; Stevens, 2002).

In addition to the findings on altered peripheral levels of estrogen in schizophrenia, evidence also indicates alterations in the brain’s response to these hormones. Both men and women with schizophrenia show reduced mRNA levels of ERα in hippocampus (Perlman et al., 2005) and reduced mRNA levels and decreased frequency of wild-type ERα mRNA in frontal cortex (Perlman et al., 2005; Weickert et al 2008). There are several single nucleotide polymorphisms (SNPs) that have been associated with schizophrenia. Weickert et al., (2008) found eighteen splice variants of ERα, one of which encoded a premature stop codon. This variant produced a truncated version of ERα that lacked the majority of its estrogen binding domain. This receptor acted as a dominant negative version which attenuated gene expression at estrogen response elements (EREs), but had no estrogen signaling function. The altered forms of ERα mRNA have been found to work as a dominant negative form of the receptor, which blocks the activity of the wild-type (Weickert et al., 2008). These studies show that the schizophrenic brain may have an attenuated response to estrogen. This along with altered blood levels of estrogen may work together to significantly blunt estrogen response in schizophrenia.

Previous studies have shown that low levels of estrogen in women with schizophrenia are correlated with increased relapse vulnerability and reduced sensitivity to antipsychotics (Arad and Weiner, 2009). A rodent study using the latent inhibition model of selective attention deficits in schizophrenia showed that estrogens are at least in part responsible for haloperidol sensitivity (Almey et al., 2013). Another study in rats showed that low levels of estrogen leads to a “pro-
psychotic state and increased resistance to typical antipsychotic treatment (Arad and Weiner, 2009). In addition to their effects on HPA axis as evidenced by changes in cortisol levels (Piriu et al., 2015; Arad and Weiner, 2009; Haddad and Wieck, 2004), antipsychotics are also known to alter estradiol levels. One study in 64 male patients who were taking either risperidone (19 subjects) or clozapine (30 subjects) and met Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria for schizophrenia or healthy controls (15 subjects) showed that both drugs reduced circulating estradiol (Piriu et al., 2015). Stimulation of ERα with 4,4′,4″-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT) and antagonism with 1,3-Bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinyloxy)phenol]-1H-pyrazole (MPP) led to a dose-dependent increase or decrease in basal prepulse inhibition (PPI), respectively in male mice (Labouesse et al., 2015). Estrogen signaling plays an important role in modulating the activity of dopamine, serotonin, GABA and glutamate, the key neurotransmitters implicated in schizophrenia. Rodent studies have revealed that estradiol increases serotonin concentrations (Sanchez et al., 2013) and enhances glutamate receptors (Meitzen and Mermelstein, 2011) and dopamine synthesis, release and turnover in cortical and striatal regions (Bethea et al., 1999; Hiroi et al., 2006; Pasqualini et al., 1995; Becker, 2000; Becker, 1990; Pecins-Thompson et al., 1996; Seeman, 1987; Xiao and Becker, 1994; Adams et al., 2004). Moreover, ovariectomy has been shown to reduce dopamine receptor 1 (D1) receptors in frontal cortex and striatum as well as D2 receptors in striatum in rats (Bossé, and DiPaolo, 1996). These changes were seen along with alterations in GABAA receptors in the substantia nigra pars reticulata, striatum, nucleus accumbens, and entopeduncular nucleus post-ovariectomy. Moreover, treatment with estradiol for 2 weeks restored the changes in D1, D2, and GABAA receptors suggesting an important role
of estrogen in neurotransmitter receptor regulation (Bosse and DiPaolo, 1996; Schotte et al., 1996).

ESTROGEN AND OTHER NEURODEVELOPMENTAL DISORDERS

Attention Deficit Hyperactivity Disorder (ADHD)

In addition to ASD and schizophrenia, estrogen has been implicated in the pathophysiology of many other neurodevelopmental disorders. Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder that occurs more often in men than women. ADHD is marked by reduced attention, hyperactivity, and impulsivity and is often mistaken for ASD. Males are twice as likely to be diagnosed with ADHD as their female counterparts (American Psychiatric Association, 2013), but the factors that contribute to the gender disparity are largely unknown. Bisphenol A (BPA) is a xenoestrogen compound that has been shown to have some effects on behavioral outcomes when children are exposed to high levels of the compound (Casas et al., 2015; Schug et al., 2015). BPA binds to estrogen receptors altering estrogen signaling and disrupting downstream effects of estrogen receptors (Schug et al., 2015). One study showed a significant association with increased BPA exposure and risk for development of ADHD-hyperactivity symptoms at 4 years of age, though this effect disappeared by 7 years of age (Casas et al., 2015). Another study showed that gestational exposure to BPA resulted in an increased risk for development of hyperactivity and aggressive behavior in children (Perera et al., 2012). The effect was stronger in girls than their male counterparts (Perera et al., 2012). Also, an aromatase gene (CYP19) variant is correlated with increased hyperactivity in boys (Miodovnik et al., 2012), suggesting alterations in the estrogen-testosterone balance has significant behavioral outcomes.
**Tourette’s Syndrome**

Tourette’s Syndrome is a neurodevelopmental disorder characterized by vocal and motor tics. The severity of the disorder peaks around puberty and shows a gender bias in which it affects 3-4 times as many males as females (Martino et al., 2013). Studies involving humans and rodents have reported that increased exposure to androgens during development can raise the risk of development of Tourette’s (Martino et al., 2013). Studies also show that tic severity in women is increased during the premenstrual reduction in estrogen levels (Martino et al., 2013; Kompoliti et al., 2001).

**Fragile X syndrome (FXS)**

Fragile X syndrome (FXS) is a genetic disorder resulting in mental retardation, hyperactivity, attention deficits, and language deficits. FXS results from an expansion of a CGG repeat in the FMR1 gene. The disorder affects both genders, but males typically show more severe symptoms due to the location of the mutation on the X chromosome. A study of the FXS mouse model has shown reduced ER$\alpha$ signaling due to the genetic mutation in FMR1 (Yang et al., 2015). Additional evidence of hormonal alterations related to FMR1 mutations has been shown in women with premutations of FMR1. Women who carry the premutation allele (which is defined as 55-200 unmethylated CGG repeats) develop hypergonadotropic hypogonadism (Sherman et al., 2014). These women stop menstruating before age 40 and show ovarian dysfunction, subfertility, and increased risk of medical conditions related to reduction in estrogen levels (Sherman et al., 2014).

**Bipolar Disorder**
Bipolar Disorder has recently been reconsidered as a neurodevelopmental disorder (Roybal et al., 2012; Sanches et al., 2008). Bipolar disorder is characterized by cycles of mania and depression, but often presents as a fairly heterogeneous disorder. Females present with symptoms later in life than men and have more rapid cycling, mixed mania, and depressive episodes than men (Arnold, 2003). Bipolar II disorder, which is more predominantly composed of depressive episodes, is also more common in women than men (Arnold, 2003). Studies have shown that reduced levels of estrogen, for example postpartum, result in increased psychosis (Arnold, 2003).

ESTROGEN AS A THERAPEUTIC TARGET IN NEURODEVELOPMENTAL DISORDERS

The above observations suggest that an optimal balance of estrogen levels and their receptors are necessary for proper brain development and function (Table 1). The profound effects of reduced estrogen signaling on ASD or schizophrenia phenotype and the potential of estrogen/estrogen receptor modulating agents to rescue behavioral deficits in rodents suggest that estrogen signaling, more specifically ERβ agonism, is a potential therapeutic target for ASD and other neurodevelopmental disorders.

As with any hormone therapy, efficacy in males and females should outweigh the risk of sexual and other side effects. When estrogen therapy is given, men can show major side effects including feminization, gynecomastia as well as other side effects such as headache and nausea (Sherwin et al., 2011). There are also concerns that estrogen therapies increase cancer risk and reduce fertility in men. A small study showed no increased risk of cancer in female to male transsexual men who were being administered estrogen therapy, but no large studies that are
applicable to the general population have been performed (Asscheman et al., 2011). For women, the risks are less pronounced, but some potentially detrimental side effects are still observed. Pre and postmenopausal women who are given estrogen therapy risk the development of venous thrombosis (Hayes et al., 2000). With both men and women showing side effects from estrogen therapy, do the benefits outweigh the risks?

Estrogen has shown effectiveness in improving verbal memory loss (Beer et al., 2006), and reducing depression, aggression, anxiety, and psychotic symptoms in men with dementia (Kyomen et al., 1999; Kyomen et al., 2002). In schizophrenia, 17β-estradiol administration has been shown to improve speech comprehension in women with schizophrenia (Bergemann et al., 2008). In contrast, Kulkarni and colleagues (2015) have shown that transdermal estradiol patch therapy in women with treatment-resistant schizophrenia significantly improves their psychotic symptoms, but no effect on cognitive function was observed (Wilk et al., 2002). In addition to estradiol, a number of studies have investigated the beneficial effects of DHEA, an intermediate in the synthesis of sex steroids, in schizophrenia. However, some studies showed improvement in attention and skill learning (Ritsner et al., 2006a; Ritsner et al., 2006b), whereas others reported no beneficial effects (Ritsner et al., 2010; Strous et al., 2007) following DHEA treatment in schizophrenia. Based on these observations, the important question here is whether a receptor-selective therapy is more beneficial than estradiol treatment? SERMs are chemicals that modulate estrogen signaling through receptors, providing a more specific therapy than estrogen administration. These molecules were developed to avoid peripheral effects of estrogen therapy while still stimulating estrogen signaling in the brain (Weickert et al., 2015). Raloxifene hydrochloride is a selective estrogen receptor modulator with mixed agonist and antagonist activity (Shang and Brown, 2002). Raloxifene has been shown to improve attention and memory,
reduce cognitive impairment, and increase learning in individuals with schizophrenia (Weickert et al., 2015; Huerta-Ramos et al., 2014; Gogos et al., 2015; Kindler et al., 2015). These outcomes have been shown to occur without feminizing effects in males. Raloxifene mimics estrogen effects on dopamine and serotonin neurotransmission, age-related attention and verbal memory loss, cognitive decline, and brain activity (Landry et al., 2002; Bethea et al., 2002; Cyr et al., 2000; Weickert et al., 2005; Goekoop et al., 2006). In a recent study, Kulkarni and colleagues (2016) have shown that Raloxifene hydrochloride treatment for 12 weeks (120 mg/d) reduces illness severity and increases the probability of a clinical response in women with refractory schizophrenia. That being said, the exact neurobiological mechanism of raloxifene has not been fully elucidated, so additional studies are warranted to prove the safety and effectiveness of this drug in long-term treatment management of schizophrenia. In particular, subjects with schizophrenia should be screened for the potential risks associated with raloxifene-induced thromboembolic events at the time of their enrolment in the study (Barrett-Connor et al., 2009; Adomaityte et al., 2008).

Very few studies have been performed to explore the efficacy of estrogen or related hormone therapies in subjects with ASD, ADHD, FXS, or Tourette’s syndrome. A few recent studies have shown that administration of tamoxifen as an adjunct to mood stabilizer medications or monotherapy improves symptoms in adults with bipolar disorder (Kulkarni et al., 2014; Yildiz et al., 2008; Talaei et al., 2016). Tamoxifen has also been shown to reduce mania and depression in children and adolescents with acute mania when added to lithium treatment (Fallah et al., 2016). The sample sizes in the above studies are small, but the results indicate some beneficial effects of tamoxifen as an effective rapid acting antimanic agent. However, the increased risk of thromboembolic events and endometrial cancer (Fisher et al., 1994; Kedar et al., 1994) limit the
potential use of tamoxifen in long-term treatment in neurodevelopmental disorders. Although tamoxifen has mixed estrogen receptor agonist and antagonist activity depending on the target tissue, it has a number of receptor-independent effects, including inhibition of protein kinase C (PKC) (O’Brian et al. 1986). Since abnormalities in PKC signaling are known in manic states (Hahn and Friedman, 1999), additional studies are warranted to address the mechanisms of action of tamoxifen in bipolar disorder.

CONCLUSIONS

As stated above, estrogen can have tremendous effects on cognition, mood, and synaptic plasticity. Estrogen receptors are most densely located in the cortex, hippocampus, and amygdala, which are regions that control cognitive function and mood. This control is highly relevant to neurodevelopmental disorders because higher order functions such as memory, learning, impulse control, and language are often impaired in these disorders. Mood is also impaired in both ASD and schizophrenia where subjects who suffer from ASD or schizophrenia show increased anxiety and depression. These factors make estrogen receptors an enticing therapeutic target for neurological and neurodevelopmental disorders. Treating with SERMs could eliminate most sexual side effects seen in treatment with estradiol or less specific estrogen modulators (Figure 1). Although SERMS have been clinically tested in a number of studies in schizophrenia subjects, their therapeutic potential in children with ASD still needs to be determined. In addition, how estrogen and estrogen receptors are developmentally dysregulated in the above neurodevelopmental disorders is largely unknown. The role of genetic and/or epigenetic mechanisms in the regulation of estrogen receptors, the effects of immune activation during development on estrogen signaling, the possible cross talk of estrogen receptors and/or
cofactors with other candidate genes implicated in ASD or schizophrenia are some other areas of research which need further investigation.
AUTHORSHIP CONTRIBUTION

Wrote or contributed to the writing of the manuscript: Crider, Pillai
REFERENCES


Arad M, and Weiner I (2009) Disruption of latent inhibition induced by ovariectomy can be reversed by estradiol and clozapine as well as by co-administration of haloperidol with estradiol but not by haloperidol alone. Psychopharmacology (Berl) 206:731–740.


women with polycystic ovary syndrome: A longitudinal case-control study. Clin Endocrinol (Oxf) 77:898–904.


Pierman S, Sica M, Allieri F, Viglietti-Panzica C, Panzica GC, Bakker J (2008) Activational effects of estradiol and dihydrotestosterone on social recognition and the arginine-


Seeman P (1987) Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1:133–152.


FOOTNOTES:

This work was supported by funding from National Institute of Health (NIH)/National Institute of Mental Health (NIMH) to Dr. Pillai [Grant R01 MH 097060].
**LEGENDS FOR FIGURES**

**Figure 1.** Schematic representation of the effects of estrogen on brain functions through estrogen receptors, ERα and ERβ. ERα activation promotes a number of cellular events including sociosexual development, activation of GABA signaling, neuroprotection and cognitive effects. ERβ activation leads to mainly neuroprotective and neurobehavioral effects. ERβ stimulation also inhibits endoplasmic reticulum stress-induced changes in mRNA transcription and protein translation via synovilin 1 dependent mechanism. Raloxifene is an agonist of both ERα and ERβ where as PPT is an ERα specific agonist. MPP is an ERα specific antagonist.
**TABLES AND FIGURES**

**Table 1.** Summary of the studies published on the effects of estrogen on various cellular functions in brain and periphery

<table>
<thead>
<tr>
<th>BRAIN</th>
<th>PERIPHERY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Memory</strong> (Kretz et al., 2004; Prange-Kiel et al., 2006)</td>
<td><strong>Spatial Memory</strong> (Rhodes et al., 2006; Khan et al., 2014)</td>
</tr>
<tr>
<td><strong>Motor Control</strong> (Martino et al., 2013; Kompoliti et al., 2001)</td>
<td><strong>Inflammation</strong></td>
</tr>
<tr>
<td><strong>Language</strong> (Whitehouse et al., 2010; Beer et al., 2006; )</td>
<td><strong>NFkB</strong> (Bebo et al 2009; Kalaitzidis and Gilmore, 2005; Evans et al., 2001; )</td>
</tr>
<tr>
<td><strong>Learning</strong> (Rissman et al., 1999; Rissman et al 2002; Weickert et al., 2015; Huerta-Ramos et al., 2014; Gogos et al., 2015; Kindler et al., 2015)</td>
<td><strong>T-cell Activation</strong> (Lee et al 2008)</td>
</tr>
<tr>
<td><strong>Neuroprotection</strong></td>
<td><strong>Endoplasmic Reticulum Stress</strong> (Kooptiwut et al., 2014)</td>
</tr>
<tr>
<td><strong>Plasticity</strong> (Hasegawa et al., 2015; Kramár et al., 2009)</td>
<td><strong>Sexual Characteristics and Reproduction</strong> (Rhodes et al., 2006)</td>
</tr>
<tr>
<td><strong>Neurotrophic factors</strong> (Yi et al., 2016; Wong et al., 2011; Solum and Handa, 2002; Golden et al., 2010)</td>
<td><strong>Puberty</strong> (Tordjman et al, 1997; Rissman et al, 2002; Shermal et al., 2015)</td>
</tr>
<tr>
<td><strong>Inflammation</strong> (Dodel et al., 1999; Drew and Chavis, 2000; Bruce-Keller et</td>
<td></td>
</tr>
<tr>
<td>Cognition (Hoff et al., 2001; Ko et al. 2006)</td>
<td>Mood (Ostlund et al., 2003; Pfaff et al., 2011; Conus et al., 2007; Kyomen et al., 1999; Kyomen et al., 2002)</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Brain Activity (Mendrek et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Social Development (Tordjman et al., 1997; Knickmeyer et al., 2006)</td>
<td></td>
</tr>
<tr>
<td>Neurotransmission</td>
<td>Dopamine (Bosse and DiPaolo, 1996; Landry et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Serotonin (Bethea et al., 2002; Amin et al. 2005)</td>
</tr>
<tr>
<td></td>
<td>Drug Response Antipsychotics (Cotton et al., 2009; Morgan et al., 2008)</td>
</tr>
</tbody>
</table>