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## Estrogen Receptors, the Hippocampus, and Memory

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### Abstract

Estradiol effects on memory depend on hormone levels and the interaction of different estrogen receptors within neural circuits. Estradiol induces gene transcription and rapid membrane signaling mediated by estrogen receptor-alpha (ER $\alpha$ ), estrogen receptor-beta (ER $\beta$ ), and a recently characterized G-protein coupled estrogen receptor, each with distinct distributions and ability to influence estradiol-dependent signaling. Investigations using receptor specific agonists suggest that all three receptors rapidly activate kinase-signaling and have complex dose-dependent influences on memory. Research employing receptor knockout mice demonstrate that ER $\alpha$  maintains transcription and memory as estradiol levels decline. This work indicates a regulatory role of ER $\beta$  in transcription and cognition, which depends on estradiol levels and the function of ER $\alpha$ . The regulatory role of ER $\beta$  is due in part to ER $\beta$  acting as a negative regulator of ER $\alpha$ -mediated transcription. Vector-mediated expression of estrogen receptors in the hippocampus provides an innovative research approach and suggests that memory depends on the relative expression of ER $\alpha$  and ER $\beta$  interacting with estradiol levels. Notably, the ability of estradiol to improve cognition declines with advanced age along with decreased expression of estrogen receptors. Thus, it will be important for future research to determine the mechanisms that regulate estrogen receptor expression during aging.

### Keywords

estrogen; aging; memory; hippocampus; estrogen receptor-alpha (ER $\alpha$ )

### Introduction

Estrogens are steroid hormones that are synthesized in the gonads as well as in various tissues throughout the body, including the brain. 17 $\beta$ -Estradiol (E2), the most potent and predominant form of estrogen, has a number of effects on cognition and brain function. Several cognitive processes appear to be dependent on the level of E2 such that cognitive

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impairment occurs when E2 concentrations are above or below an optimal level (Fig. 1) (Foster 2005; 2012). Numerous clinical research studies have examined memory and cognitive function in women whose levels of E2 have been altered either through ovariectomy or menopause. In general, the results of these studies show a decrease of cognitive function in women following surgical removal of their ovaries or menopause, and that these deficits can be reversed by estrogen replacement therapy (ERT) if the therapy is initiated immediately following ovarian hormone loss (Sherwin 2009). The observation that the effectiveness of ERT is tightly linked to the woman's physiological status at the time of initiation of treatment is echoed by the results of more recent studies that show that the cognitive benefits of ERT are minimal in women older than 65 years (Hogervorst and Bandelow 2010; Maki and Henderson 2012). Indeed, the effectiveness of ERT to reduce risk of Alzheimer's disease in menopausal women is lost when treatments are initiated in women of advanced age (Brinton 2001; Resnick and others 1997; Zandi and others 2002).

Much of what we currently know about the mechanisms for estrogen's effects on cognitive function comes from studies of learning and memory in rodent models, the results of which parallel those observed in women. For example, the ability of E2 to improve performance on spatial memory learning tasks in ovariectomized rodents is highly dependent on the age of the animal, the dose of E2, and the duration of estrogen decline prior to initiation of E2 treatments. In these studies, E2 treatment was observed to improve spatial memory in young (3 months) ovariectomized rats when the treatments were initiated at middle-age but not when begun at later ages (Foster and others 2003; Gibbs 2000; Markham and others 2002; Markowska and Savonenko 2002). However, when animals were ovariectomized in middle-age (12-13 months), treatments initiated at 17 to 21 months did not improve spatial working memory (Daniel 2006; Gibbs 2000; Markowska and Savonenko 2002). The reduced ability of E2 to improve memory in these rat studies mirrors ERT clinical observations and indicates that the underlying mechanism(s) that account for the effectiveness of ERT to improve memory performance in women are likely to be multifaceted and influenced by age and the duration of hormone deprivation in each individual.

E2 influences brain function through gene transcription and rapid membrane signaling. Furthermore, several transcriptional and post-translational feedback mechanisms control E2 activity, which likely contributes to the complex dose-response pattern. While the mechanism(s) underlying the reduced ability of ERT to provide cognitive protection with advancing age is unknown, it is known that E2 effects on several biological processes decline with age, including the ability to preserve the blood-brain barrier (Bake and Sohrabji 2004), protect the brain from stroke (Suzuki and others 2009) and neuroinflammation (Benedusi and others 2012), promote growth of glutamatergic synapses (Adams and others 2001; Aenlle and Foster 2010), influence mitochondrial function (Aenlle and Foster 2010; Jones and Brewer 2009), and activate rapid signaling cascades (Fan and others 2010; Foster 2005; Wu and others 2013).

The purpose of this review is to highlight our current understanding of how E2 signaling may affect hippocampal-dependent cognitive function. We begin with a brief overview of rapid E2 signaling mechanisms and the classical nuclear E2 pathways, with an emphasis on the role of the different estrogen receptors. The second section explores the role of estrogen

receptors in the regulation of cognition by examining studies of spatial memory that employ receptor-specific agonists, receptor knockout mice, and viral vectors to overexpress receptors in specific hippocampal cells. Finally, we examine how changes in estrogen receptor expression interact with the level of E2 to determine cognitive decline during aging and discuss the mechanisms for regulation of estrogen receptor expression. The data indicate that the various receptors can have similar or antagonistic effects and the influences on memory are because of the interaction of estrogen receptors, which in turn depend on their level of expression and the level of agonist.

## Estrogen Receptor Signaling Mechanisms

E2 is thought to exert the majority of its biological effects through the interaction of two primary estrogen receptors: estrogen receptor-alpha (ER $\alpha$ ) and estrogen receptor-beta (ER $\beta$ ). In addition, a G-protein coupled estrogen receptor (GPR30 or GPER1) has recently been characterized as a modulator of second messenger pathways. The relative levels and subcellular distributions of ER $\alpha$ , ER $\beta$ , and GPER1, which are found throughout the nervous system, vary across brain regions (Brailoiu and others 2007; Milner and others 2001; Mitra and others 2003; Mitterling and others 2010). In human and rat hippocampus, ER $\beta$  levels are greater than ER $\alpha$  levels (Foster 2012) and ER $\alpha$  is more likely to be localized to the nucleus (Mitra and others 2003; Mitterling and others 2010). Because of differences in structure and subcellular distribution, it has been argued that estrogen receptors may have different biological actions and differential effects on cognition.

### Rapid Signaling Mechanisms

Rapid signaling is mediated through membrane receptors, including the GPER1 G-protein coupled receptor, and the interaction of ER $\alpha$  and ER $\beta$  with metabotropic receptors (Fig. 2A). These membrane receptors initiate Ca<sup>2+</sup>, phospholipase C, and adenylyl cyclase signaling with subsequent activation of a host of kinases (B-RAF, IP3K, Src, ERK, AKT, PKA, PKC), which in turn can rapidly influence neuronal physiology or phosphorylate transcription factors such as CREB or ER $\alpha$  to induce gene transcription (Foster 2005). As such, E2 signaling interacts with molecular pathways associate with growth factor signaling as well as learning and memory. Time course and dose–response studies indicate that kinase activity rapidly rises and declines within minutes and activation exhibits an inverted U function, with inhibition at higher doses (Kuroki and others 2000).

Research using receptor-specific agonists indicates that membrane ER $\alpha$  and GPER1 initiate AKT cell survival signaling and all three receptors can initiate Ca<sup>2+</sup> to ERK signaling (Boulware and others 2013; Gingerich and others 2010; Maruyama and others 2013; Wu and others 2011; Zhang and others 2009), which is thought to be involved in cell survival and modulation of memory. Similarly, work with estrogen receptor knockout (ERKO) mice indicates that multiple estrogen receptors contribute to rapid effects of E2 on synaptic transmission (Foster and others 2008; Fugger and others 2001). Finally, in an elegant series of studies, it was shown that the rapid kinase signaling cascades enhanced ER $\alpha$ -mediated transcription through the phosphorylation of ER $\alpha$  (Clark and others 2013). Thus, for hippocampal cells that express both genomic and membrane receptors, the specificity of action of ER $\alpha$  and ER $\beta$  on rapid signaling cascades may be mute if all receptors activate the

same rapid signaling systems and ultimately contribute to ER $\alpha$ -dependent transcription (Fig. 2A).

### Classical Nuclear Pathways for Regulating Transcription

Considering the diverse assortment of hippocampal molecular and biological processes influenced by E2, it is not unanticipated that E2 treatment regulates the expression of hundreds of hippocampal genes (Aenlle and Foster 2010; Aenlle and others 2009; Han and others 2013). Receptor specific agonists have been employed to examine the possible role of ER $\alpha$  and ER $\beta$  on biological processes that depend on transcription and likely contribute to cognitive function during aging. Activation of ER $\alpha$  or ER $\beta$  differentially influences the expression of neuroprotective genes and neuroprotection to ischemia and  $\beta$ -amyloid protein (Carroll and Pike 2008; Farr and others 2007; Gingerich and others 2010). E2 increases the expression of genes linked to the cellular components of the synapse (Aenlle and Foster 2010). Both receptors appear to influence synaptogenesis, but most studies using receptor-specific agonists find that synaptic markers are increased more by ER $\alpha$  activation, relative to the ER $\beta$  agonists, suggesting that ER $\alpha$  is the predominate receptor for synaptogenesis (Foster 2012). Interestingly, the extent of synaptogenesis may depend on the relative expression of ER $\alpha$ /ER $\beta$ , with a decrease in synaptogenesis observed as ER $\beta$  levels increase (Szymczak and others 2006).

Despite the fact that ER $\beta$  is the predominately expressed receptor in the hippocampus, ER $\alpha$  is transcriptionally more active than ER $\beta$  (Fig. 2B). Although both receptors exhibit similar homology for the DNA binding domain, the difference in transcriptional activity is, in part, because of the structural differences that influence the affinity of the receptor for E2 and interactions with transcription activators/repressors (Barkhem and others 1998) (Fig. 3). The consequence of these structural differences is that low levels of E2 will preferentially activate ER $\alpha$ -dependent transcription (Foster 2012). However, for low E2 levels, ER $\beta$  can heterodimerize with ER $\alpha$  to act as a negative regulator of ER $\alpha$ -mediated transcription (Pettersson and others 2000). Co-expression of both receptors in cell cultures results in an ER $\beta$  gene-dose inhibition of transcription (Hall and McDonnell 1999; Pettersson and others 2000). Thus, the higher level of ER $\beta$  in the hippocampus may act to inhibit ER $\alpha$ -dependent transcription (Fig. 2B). Indeed, some ER $\beta$  isoforms found in the hippocampus act as dominant negative regulators of ER $\alpha$ -dependent transcription (Chung and others 2007). Thus, in many instances, ER $\beta$  has a role in regulating ER $\alpha$  activity.

ER $\beta$  regulation of ER $\alpha$ -mediated transcription is particularly evident in mice with a functional knockout of ER $\alpha$  or ER $\beta$ . Relative to wildtype controls, ER $\alpha$ -mediated transcription is increased in tissues from ER $\beta$ KO mice (Foster 2012). Recent work (Han and others 2013) employing mutant mice revealed that, in the hippocampus, E2-related transcription was observed in ovariectomized ER $\beta$ KO mice in the absence of E2 treatment (Box 1). The results indicate that ER $\alpha$ -mediated transcription can occur under conditions of low E2 and confirms an inhibitory role for ER $\beta$  (Fig. 4A). Interestingly, a single E2 treatment decreased expression of E2-sensitive genes in ER $\beta$ KO mice suggesting that feedback mechanisms may attempt to limit over activation of ER $\alpha$  following E2 treatment. Finally, a single injection of E2 resulted in E2-dependent transcription in ER $\alpha$ KO mice,

suggesting that other E2 sensitive mechanisms, possibly involving a transcriptionally weaker ER $\beta$ , can induce transcription and maintain hippocampal function provided E2 levels are increased (Fig. 4B).

## Estrogen Receptors in Cognition

The various estrogen receptors may differentially contribute to time-dependent and dose-dependent effects of E2 treatments on cognition. For example, E2 is thought to influence memory through estrogen receptors in the membrane that rapidly activate signaling cascades involved in synaptic plasticity processes that are required for learning and memory (Fig. 2A). In this case, E2-mediated enhancement of memory is observed when treatment is delivered around the time of training, but not when it is delayed by 2 hours (Packard 1998). Long-term effects are thought to result from transcription of genes that maintain cognitive function such that memory facilitation is observed several days after an E2 treatment. In this way, cyclic E2 treatment improved memory in middle-age mice 48 to 72 hours after E2 treatment (Aenlle and others 2009; Han and others 2013). Finally, difference may be observed for cyclic and chronic treatments, possibly because of feedback mechanisms (Gibbs 2000; Markowska and Savonenko 2002).

The relationship between the E2 dose and memory can be described by an inverted U function, such that memory improvement is supplanted by impaired memory as E2 levels increase (Foster 2012) (Fig. 1). E2 induces a similar biphasic response in a wide range of tissues and biological processes (Calabrese 2001). In other receptor systems, receptor subtypes contribute to a biphasic dose response in rapid signaling due to differences in receptor affinity and the effectiveness of signal transduction for receptor subtypes with antagonistic actions. As noted above, E2-mediated transcriptional activity is a function of differences in ligand affinity and the efficacy of transcription driven by homodimers and heterodimers suggesting one possible mechanism for the inverted U dose–response.

## Receptor Specific Agonists

In an attempt to examine the role of each receptor subtype in memory, recent work has employed receptor specific agonists, mutant mice, and viral vector delivery of different estrogen receptors. The results suggest a framework for thinking about how the various estrogen receptors interact with the level of E2 to influence memory. For studies on the role of each receptor in rapid effects in memory modulation, receptor specific agonists are delivered within a relatively short time prior to or just after a learning episode. For example, post-training treatment with the ER $\alpha$ -specific agonist, propyl pyrazole triol (PPT, 0.9 mg/kg), but not the ER $\beta$ -specific agonist, diarylpropionitrile (DPN, 0.9 mg/kg) improved retention on the object placement task in 2- to 4-month ovariectomized rats (Frye and others 2007). Interestingly, the opposite effect was observed by another group (Jacome and others 2010), which used a higher dose (3-5 mg/kg) and found that DPN, but not PPT, improved memory in 2-month ovariectomized rats. One study has specifically examined the dose–response function for ER $\alpha$ - and ER $\beta$ -specific agonists on the object placement task (Phan and others 2011). The results indicated that in 2-month ovariectomized mice, PPT treatment prior to training resulted in an inverted U function for memory and hippocampal dendritic spine number. In contrast, DPN had only a modest effect on spatial memory and no effect on

spine number. The differences emphasize the complex dose–response relationship for estrogen receptor agonists and the need to examine multiple doses.

Long-term treatment with PPT, DPN, or the GPER1 agonist G-1 improved delayed matching-to-position on a T-maze in young rats (Hammond and others 2009), suggesting a lack of receptor specificity for long-term effects on this task. Because the activation of membrane ER $\alpha$ , ER $\beta$ , or GPER1 receptors lead to activation of similar kinase pathways, ER $\alpha$  phosphorylation, and ER $\alpha$ -mediated transcription (Fig. 2A), it will be important for future studies to determine the mechanism for improved cognition. Several studies have examined the effects of chronic ER $\alpha$ - or ER $\beta$ -specific agonists on spatial memory in middle-age animals. In one study, rats were ovariectomized at 3 months of age and treatment with the ER $\alpha$  agonist PPT (1 mg/kg) was initiated at ~5 months. Animals were tested after 2 months of treatment and exhibited improved spatial learning and memory on the water maze, which was associated with neuroprotection and preservation of synaptic function (Qu and others 2013). The dose–response (0.02–0.2 mg/kg) influence of PPT and DPN was examined in middle-age (12 months old) ovariectomized rats on a delayed spatial alternation task (Neese and others 2010). This study reported that the lowest dose of DPN impaired performance and intact performance was observed for higher doses. In contrast, PPT had only subtle effects at the highest dose. Together, the studies emphasize that, similar to acute studies, chronic studies of receptor specific agonists should employ multiple doses to determine the dose–response function. Furthermore, for chronic studies, it will be important to determine which mechanisms, rapid activation of kinases and/or ER $\alpha$ -mediated transcription, mediate the preservation of cognitive function during aging.

### Estrogen Receptor Knockout Mice

Other studies have attempted to differentiate cognitive effects of estrogen receptor subtypes through receptor knockout mice. In many ways, these studies mirror the results of hippocampal transcription in receptor knockout mice, indicating that ER $\alpha$  maintains hippocampal function, particularly when E2 levels are low, and ER $\beta$  can have an inhibitory effect on ER $\alpha$ -mediated effects. In the absence of E2 treatment, ovariectomized young and middle-age ER $\alpha$ KO mice exhibit impaired memory (Foster and others 2008; Fugger and others 2000; Han and others 2013), supporting a role for ER $\alpha$  in maintaining cognitive function. The results are consistent with a growing literature that indicates that memory function is positively correlated with hippocampal ER $\alpha$  levels and, under conditions of low E2, cognitive deficits are observed when ER $\alpha$  function is impaired (Foster 2012). In women, some ER $\alpha$  polymorphisms are associated with an increased susceptibility to Alzheimer's disease (Corbo and others 2006; Ji and others 2000). Interestingly, ER $\alpha$  polymorphisms are also associated with an increased propensity for cognitive impairments, which emerge as E2 levels decline during middle-age (Olsen and others 2006; Yaffe and others 2002). The results suggest that prior to menopause, elevated levels of E2 may be able to compensate for impaired ER $\alpha$  function. This idea was confirmed in ER $\alpha$ KO mice in which E2 treatment improved memory, suggesting that other estrogen sensitive mechanisms, possibly involving ER $\beta$ , may be engaged by elevation of E2 to compensate for a decrease in ER $\alpha$  function (Fugger and others 2000; Han and others 2013; Liu and others 2008).

Research using ER $\beta$ KO mice indicates that the role of ER $\beta$  in cognition depends not only on the level of E2 but also on the function of ER $\alpha$ . Unlike ER $\alpha$ KO mice, ovariectomized ER $\beta$ KO mice do not exhibit an initial impairment in memory (Fugger and others 2000; Han and others 2013). In fact, in middle-age mice and young mice, 6 weeks following ovariectomy, cognition of ER $\beta$ KO mice is enhanced relative to both ER $\alpha$ KO and wild-type mice (Han and others 2013). The contrast between wild-type and ER $\beta$ KO mice suggests that, when E2 levels are low, ER $\beta$  expression has an antagonistic effect on cognition. This effect of ER $\beta$  is thought to result in part from ER $\beta$  acting as a negative regulator of ER $\alpha$ -mediated transcription. The antagonistic action of ER $\beta$  may also protect animals from cognitive impairment because of overactivation of ER $\alpha$ . In ER $\beta$ KO, but not wild-type mice, chronic E2 treatment resulted in a dose-dependent impairment of cognition (Fig. 4A) and a decrease in ER $\alpha$  expression (Rissman and others 2002), suggesting overactivation of ER $\alpha$  and subsequent feedback regulation. Finally, ER $\beta$ KO mice do not exhibit the memory-enhancing effects of acute E2 treatment normally observed in wildtype and ER $\alpha$ KO mice (Fugger and others 2000; Han and others 2013; Walf and others 2008). Together, the results demonstrate that ER $\alpha$  maintains cognitive function in the face of declining E2 levels and that ER $\beta$  can be antagonistic to memory for low E2 levels (Fig. 4), suggesting that dose-dependent effects of E2 on cognition may depend on the relative expression of ER $\alpha$  and ER $\beta$ .

### **Viral Vector Delivery of ER $\alpha$ and ER $\beta$**

Viral-mediated delivery of estrogen receptors provides a novel approach to determine the role of estrogen receptor expression in cognitive function. This technique has the advantage of enhancing receptor function in specific brain regions and cell types, and at specific ages. Vector delivery of ER $\alpha$  to neurons of the hippocampus rescues memory function in adult ovariectomized ER $\alpha$ KO mice (Foster and others 2008). The results indicate that memory impairments of ER $\alpha$ KO mice are not simply because of the absence of ER $\alpha$  during development; rather, ER $\alpha$  is important in maintaining cognitive function in adults. This idea was confirmed for middle-age rats in a study that showed that, in the absence of exogenous E2, viral vector-mediated expression of ER $\alpha$  in pyramidal cells of the hippocampus improved spatial working memory (Witty and others 2012). It is important to note that in all cases, memory enhancement was observed in the absence of E2 treatment suggesting that an increase in the ER $\alpha$  expression can preserve cognitive function in adults, at least up through middle-age. In contrast, delivery of a viral vector to increase expression of ER $\beta$  in neurons of the hippocampus reverses the protective effect on cognition observed in middle-age ER $\beta$ KO mice (Han and others 2013). It is unclear if the cognitive impairment associated with delivery of ER $\beta$  reestablishes the ability of E2 to improve cognition. Regardless, the results confirm that ER $\beta$  expression can contribute to impaired cognition associated with E2 deprivation.

Together, the results suggest that the dose-dependent effects of E2 on cognition are linked to the relative expression of ER $\alpha$  and ER $\beta$  (Foster 2012). A decrease in the expression of ER $\beta$  (e.g., ER $\beta$ KO mice) results in a leftward shift in the descending arm of the dose-response curve observed as improved cognition and increased ER $\alpha$ -mediated transcription for ovariectomized animals (i.e., low E2 conditions). Furthermore, with a loss of ER $\beta$ , acute E2

treatments fail to improve memory and may activate feedback systems to decrease transcription. Moreover, chronic E2 treatment impairs memory, possibly because of a loss of ER $\beta$ -mediated regulation of ER $\alpha$  (Fig. 4A). In the case of impaired ER $\alpha$  function (e.g., ER $\alpha$ KO mice), the ascending arm of the dose response function is shifted to the right. Impairments are more profound for ovariectomized animals and an increase in E2 level can promote transcription and cognitive function (Fig. 4B).

## Estrogen Receptors and Age-Related Cognitive Decline

While the exact reason for the loss of the cognitive enhancing effects of E2 with advanced age is unknown, evidence suggests it is linked to age-related changes in expression and signaling of the estrogen receptors (see Foster 2012 for review). Gene profiling studies indicate that aged animals are less responsive than young and middle-age animals, exhibiting reduced E2-induced hippocampal transcription (Aenlle and Foster 2010). Interestingly, the decreased responsiveness was observed for genes that decline with advancing age, including genes linked to transcriptional regulation, growth, synaptic activity, and neuroprotection (Box 2). Similarly, loss of ER $\alpha$  due to extended E2 deprivation (Zhang and others 2009) or in ER $\alpha$ KO mice (Suzuki and others 2009) is linked to a loss of E2-mediated neuroprotection and synaptogenesis. Together, the results suggest that a decline in ER $\alpha$  expression may contribute to decreased E2 responsiveness.

There is a significant decrease in the number of estrogen receptor positive neurons in the hippocampus during aging. For example, there is a 56% and 41% decrease of ER $\alpha$  and ER $\beta$  respectively in the CA1 region of aged rats compared to young rats (Mehra and others 2005). Interestingly, this decrease in hippocampal estrogen receptors is not reflected in the uterus, which remains responsive to estrogen treatment. There is also a 50% reduction in the number of spines/synapses that contain ER $\alpha$  in the rat CA1 region of the hippocampus and the ability of estrogen treatment to induce spine formation declines with age and is associated with this loss of ER $\alpha$  (Adams and others 2002). Like ER $\alpha$ , the level of ER $\beta$  at the synapse decreases with age, but the level of synaptic ER $\beta$  increases following E2 treatment (Waters and others 2011). These observations suggest that changes in mechanisms for regulation of estrogen receptor expression contribute to the shift in the relative expression of ER $\alpha$  and ER $\beta$ . Thus, in older animals a decline in ER $\alpha$  and an E2-mediated increase in ER $\beta$  would result in a decrease in the ratio of ER $\alpha$ /ER $\beta$ . As noted above, a decrease in the relative expression of ER $\alpha$ /ER $\beta$  due to loss of ER $\alpha$  or an increase in ER $\beta$  is associated with a decrease in transcription of neuroprotection and synaptic plasticity genes (Aenlle and Foster 2010; Aenlle and others 2009; Han and others 2013) and reduced synaptogenesis (Szymczak and others 2006). The results suggest that the expression of ER $\alpha$  and ER $\beta$  is important for maintaining hippocampal function with advancing age.

## Regulation of Estrogen Receptor Expression

While the expression profile for estrogen receptors is well characterized by age and brain region, the molecular mechanisms that regulate estrogen receptor expression in the hippocampus are not well understood. Experiments to examine regulation of estrogen receptor expression have mainly been performed in breast cancer cell cultures. Because of their importance in regulating numerous genes and physiological processes, several



transcriptional and post-translational feedback mechanisms control estrogen receptor expression. Post-translational mechanisms include protein down-regulation via the ubiquitin–proteasome pathway (Fig. 5). ER $\beta$  binds to the heat shock cognate protein 70 (Hsc70)-interacting protein (CHIP) and induces degradation via ubiquitination and proteolysis. The degradation of ER $\beta$  results in a decrease in ER $\beta$ -mediated transcription (Tateishi and others 2006). In addition, degradation of ER $\alpha$  through the ubiquitin–proteasome pathway has been reported in the CA1 region of the rat hippocampus (Zhang and others 2011). In this study, an increase in ER $\alpha$ –CHIP interaction was observed in ovariectomized young rats and intact aged female rats. However, it is important to note that unlike ER $\beta$ , cycling of ligandbound ER $\alpha$  through the ubiquitin–proteasome degradation pathway is required for ER $\alpha$ -mediated transcription (Lonard and others 2000) (Fig. 5). Interruption of ER $\alpha$  ubiquitination and proteolysis will stabilize the receptor in the nuclear matrix, resulting in inhibition of transcription. Thus, it will be important for future studies to determine if the increase in ER $\alpha$ –CHIP interaction indicates increased or decreased ER $\alpha$ -mediated transcription.

The mRNA for ER $\alpha$  exhibits a relatively long 3'-untranslated region (3'UTR), which makes it subject to post-transcriptional regulation by binding of proteins and small noncoding microRNA (miRNA) (Fig. 5). Expressions of miR-18a, miR-22, miR-206, miR-221, and miR-222 are associated with decreased ER $\alpha$  expression in human cancer cell lines. Interestingly, miR-206 expression was inhibited by PPT, but not DPN, suggesting a reciprocal inhibitory feedback loop. However, it appears that the levels for known miRNAs that regulate ER $\alpha$  expression do not change in the hippocampus with E2 treatment or with age (Rao and others 2013).

A number of epigenetic mechanisms can produce stable changes in transcription and several epigenetic mechanisms for regulating estrogen receptor transcription have been described (Fig. 6). Histones are proteins involved in the packaging and unwinding of DNA regions through chemical modifications, including histone acetylation. Histone deacetylation is associated with silent chromatin and in breast cancer cells, a loss of ER $\alpha$  expression can be reversed by inhibition of histone deacetylase. Again, a mutual inhibitory feedback system could be in play since E2 treatment decreases hippocampal expression of genes (HDAC2, Sap18) involved in histone deacetylation in young and middle-age mice, but not aged mice (Aenlle and Foster 2010). Repression of gene expression can also occur through methylation of cytosines in guanine-cytosine-rich areas of the gene promoter region, termed CpG islands (Fig. 6). In several systems, ER $\alpha$  gene promoter methylation rises with age and is associated with decreased ER $\alpha$  expression and increased incidence of disease, particularly in cancer where it has been studied in depth (Li and others 2004; Post and others 1999). Similarly, methylation of the ER $\beta$  promoter was found in atherosclerosis, a common condition in vascular aging, and in senescence of smooth muscle and endothelial cell lines. The epigenetic mechanism was confirmed in the cell lines by the exposure of a DNA demethylating agent and histone deacetylase, which caused the recovery of ER $\beta$  expression (Kim and others 2007). In the brain, methylation of the ER $\alpha$  promoter during development results in a decrease in ER $\alpha$  expression and contributes to lifelong physiological and behavioral differences (Gore and others 2011; Schwarz and others 2010). Little is known concerning estrogen receptor promoter methylation in the hippocampus during aging.

However, modifications in methylation of ER $\alpha$  and ER $\beta$  promoters have been reported in the cortex of middle-age rats and following brain injury (Westberry and others 2011). The results point to promoter methylation as an important, but relatively unexplored, regulator of estrogen receptor expression in the brain across the lifespan.

Finally, estrogen receptor expression is controlled by ER $\alpha$  promoter activity, which can be regulated by mitogen and growth factor signaling pathways, as well as through other nuclear receptors, including auto-regulation through ER $\alpha$  (Pinzone and others 2004). Interestingly, E2 treatment within the physiological range, promotes expression of ER $\alpha$  in the hippocampus, while supraphysiological levels result in a decrease in ER $\alpha$  expression indicating tightly controlled feedback mechanisms (Foster 2012). However, long-term E2 deprivation or the loss of locally synthesized E2 results in a decrease in ER $\alpha$  expression and impairment in the ability of E2 treatment to increase ER $\alpha$  expression (Bohacek and Daniel 2009; Prange-Kiel and others 2003). In one study, 40 days of E2 treatment, initiated in middle-age rats, provided long-term enhancement of memory into old age that coincided with increased ER $\alpha$  levels for up to 8 months following termination of treatment (Rodgers and others 2010). Another experiment saw a significant increase in ER $\alpha$  levels in the hippocampus of rats ovariectomized at 15 months of age and treated with E2 for 10 days; however, there was no significant increase in ER $\alpha$  levels when animals were ovariectomized at 10 months of age and the E2 treatment was delayed for 5 months, suggesting that a prolonged delay in E2 therapy triggers mechanisms to render ER $\alpha$  expression insensitive to E2 treatment (Bohacek and Daniel 2009). It is important to note that in the above experiments there was no change in ER $\beta$  levels in the rat hippocampus. Together, these results imply that reduced expression and/or function of ER $\alpha$  with age or ovariectomy may be a critical factor in mediating the decrease ability of E2 to promote memory with advancing age.

## Conclusion

In summary, work using receptor specific agonists, mutant mice, and viral vector delivery of different estrogen receptors has revealed a framework for thinking about the role of estrogen receptors in memory. This work indicates that ER $\alpha$ , ER $\beta$ , and GPER1 interact to promote or inhibit signaling. Membrane ER $\alpha$ , ER $\beta$ , and GPER1 receptors initiate similar signaling cascades that activate kinases involved in modulating memory, including initiating ER $\alpha$ -mediated transcription through phosphorylation of ER $\alpha$ . Genomic estrogen receptor activity induces the transcription of genes involved in memory and maintenance of hippocampal function, including genes for rapid signaling cascades, neuroprotection, and neuronal plasticity. When E2 levels are low, ER $\beta$  can act as a negative regulator of ER $\alpha$ -mediated transcription, such that an elevation of E2 (i.e., ERT) may be required to obtain optimal cognitive function. Recent work using gene delivery indicates that shifting the relative expression of ER $\alpha$ /ER $\beta$ , to favor ER $\alpha$ , preserves cognition in the face of declining E2 levels during menopause. With advancing aging, a decrease in the relative expression of ER $\alpha$ /ER $\beta$ , mainly due to a loss of ER $\alpha$ , is associated with a loss of E2 responsiveness. It is clear that ER $\alpha$  expression decreases in the hippocampus with advanced age; however, the molecular mechanisms that mediate the decrease in ER $\alpha$  remain to be elucidated.

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**Box 1**

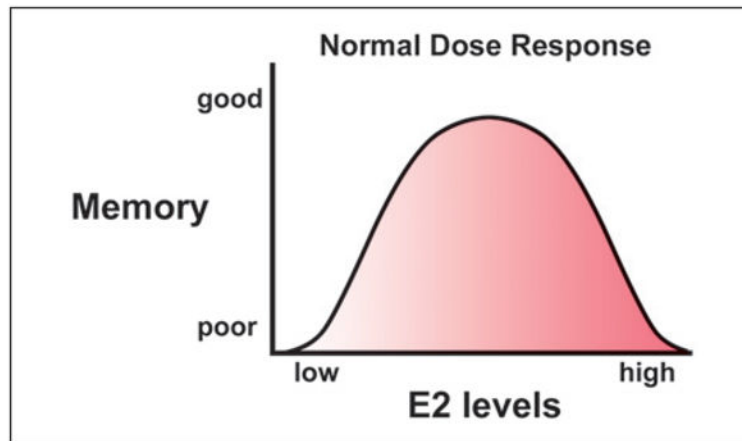
How can ER $\alpha$  induce transcription in ovariectomized animals in the absence of E2 treatment? E2 is synthesized in the hippocampus and the level of synthesized E2 is within the range that preferentially binds and activates ER $\alpha$  (Hojo and others 2004; Prange-Kiel and others 2003). However, an extended period of hormone deprivation results in the loss of locally synthesized E2 (Barker and Galea 2009). In turn, a decrease in local E2 synthesis results in a decrease in ER $\alpha$  expression. Similarly, a loss of E2 synthesis and ER $\alpha$  is observed in Alzheimer's disease (Ishunina and Swaab 2007). The results emphasize the interactions of feed forward and feedback mechanisms such that the local level of E2 interacts with ER $\alpha$  and ER $\beta$  expression to determine transcriptional activity, including the transcription of ER $\alpha$ . The interaction of E2, ER $\alpha$ , and ER $\beta$  may provide a mechanism to insure proper transcription and maintenance of hippocampal function as E2 levels fluctuate across the estrus cycle in adults (Foster 2012; Han and others 2013). However, this same interaction may also contribute to impaired cognition following the loss of ovarian hormone and a decline in ER $\alpha$  expression during menopause.



**Box 2**

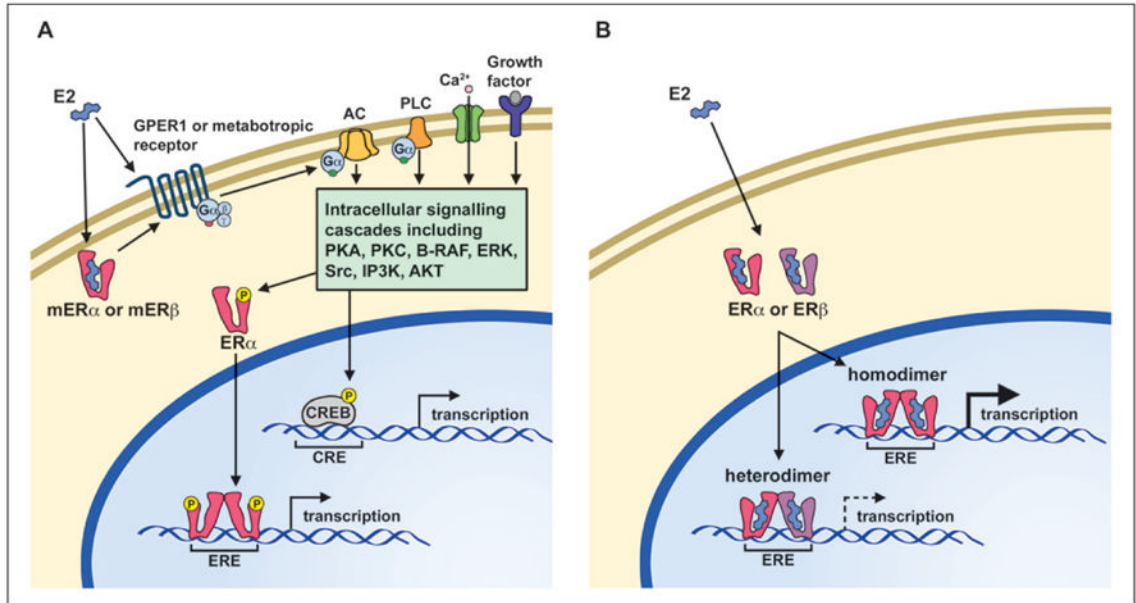
Interaction of Aging and E2 on Transcription		
	Aging	E2
Neuroprotection	↓	↑
Structure and Growth	↓	↑
Inflammation and Stress	↑	↓
Transcription Repressors	↑	↓
Synaptic Plasticity	↓	↑

The effect of aging and E2 treatment on hippocampal genes associated with specific biological responses. Aging is associated with an increase in expression of genes associated with transcription repression, inflammation and stress, and a decrease in expression of genes associated with neuroprotection, structure and growth, and synaptic plasticity. E2 treatments can reverse the age-related expression profile.

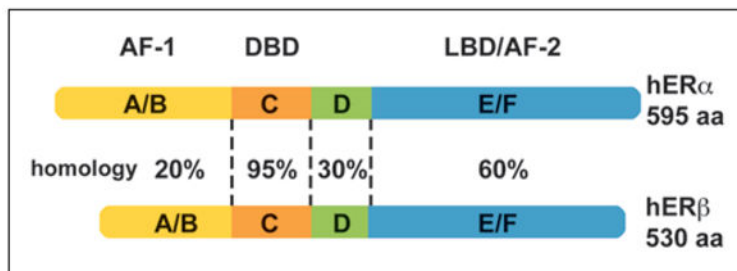


**Figure 1.**

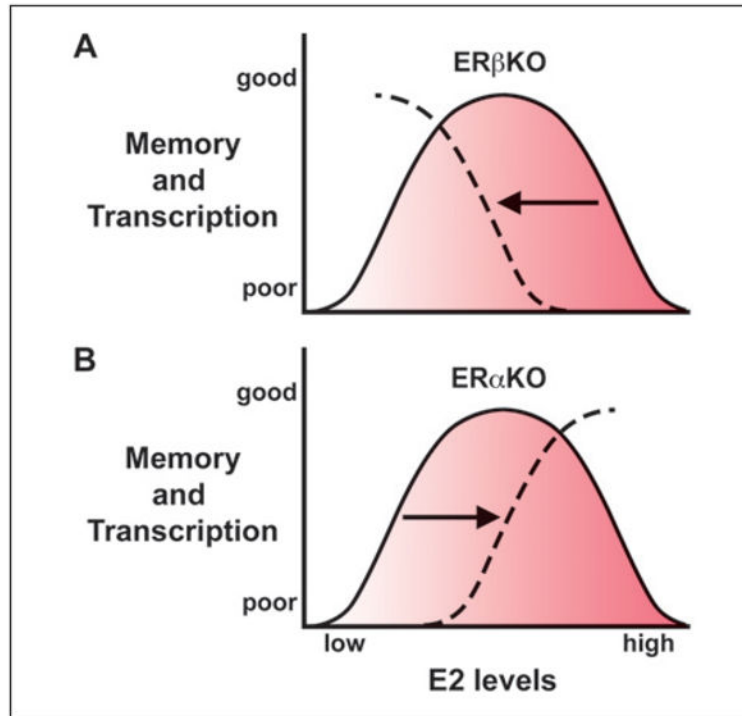
Dose–response function for  $17\beta$ -estradiol (E2) effects on cognition. The relationship between the E2 dose and memory can be described by an inverted U function, such that ovariectomy and a low level of E2 result in poor memory. Memory is enhanced by increasing E2 to within the physiological range. Memory improvement is supplanted by impaired memory as E2 levels increase to supraphysiological levels.



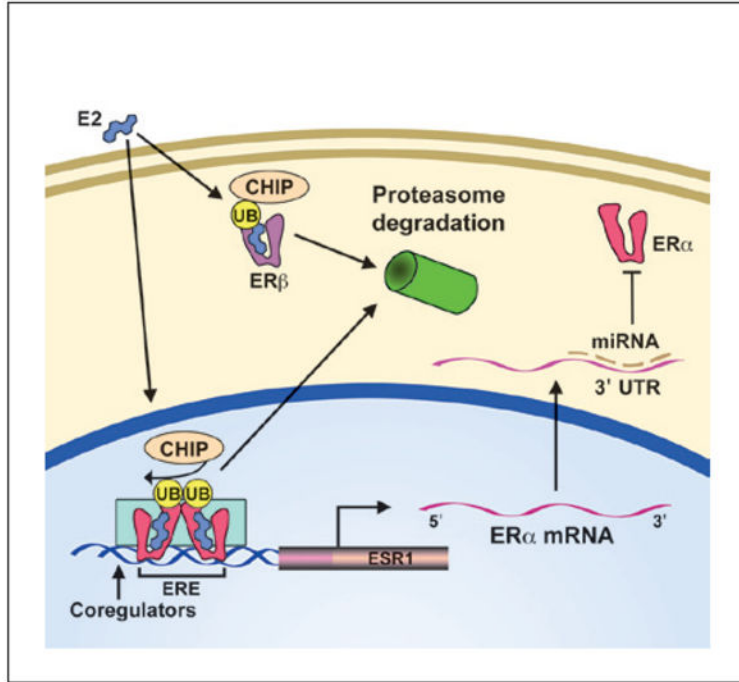
**Figure 2.** Simplified models depicting rapid estrogen receptor signaling (left) and classical nuclear pathway (right). (A) In the rapid estrogen receptor signaling pathway, estrogen activates membrane associated estrogen receptors and associated G-protein coupled receptors to induce intracellular signaling cascades, which rapidly influence neuronal physiology or lead to the phosphorylation of the estrogen receptor-alpha (ER $\alpha$ ) or CREB proteins. (B) In the classical pathway, estrogen binds to an inactive ER $\alpha$  or ER $\beta$  within the cytoplasm or the nucleus of the cell. The activated estrogen receptor monomer forms a dimer with another activated estrogen receptor monomer to create either a homodimer or a heterodimer, which then binds to an estrogen response element (ERE) within the DNA, along with other co-regulator proteins (not shown) to modify transcription of target genes. Note that ER $\alpha$  homodimers exhibit increased transcription relative to estrogen receptor heterodimers or ER $\beta$  homodimers because of ER $\beta$  acting as a negative regulator of ER $\alpha$ -mediated transcription.



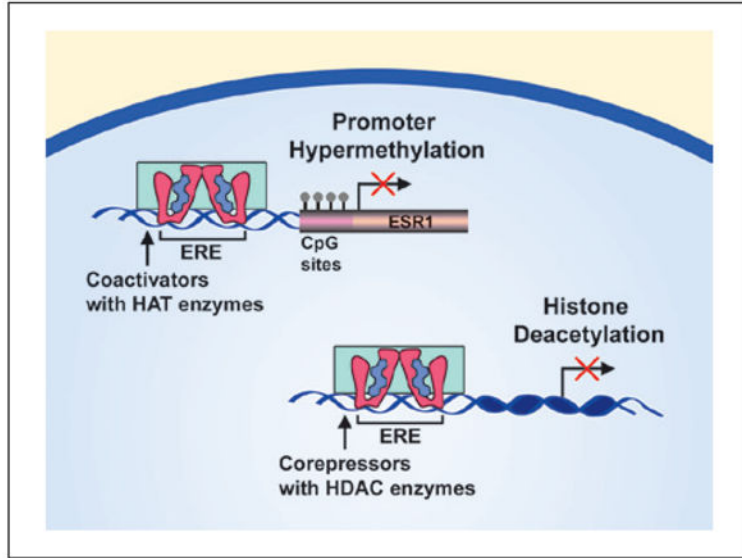
**Figure 3.** Non-homologous domains/regions within human nuclear estrogen receptors (hERs) contribute to dissimilarities in transcription. Although ER $\alpha$  and ER $\beta$  are highly homologous (95%) within the DNA binding domain (DBD), ER $\alpha$  has greater transcriptional activity. Differences in the ligand binding domain (LBD) contribute to increased affinity of 17 $\beta$ -estradiol (E2) for ER $\alpha$ . Furthermore, differences in the structure of the activation function regions (AF-1 and AF-2) influence the ability of transcriptional co-regulators to interact with the receptors.



**Figure 4.** Role of estrogen receptor-alpha ( $ER\alpha$ ) and  $ER\beta$  in transcription and cognition: studies using ERKO mice. (A) For  $ER\beta$ KO mice, the absence of  $ER\beta$  results in a leftward shift in the descending arm of the dose–response curve. Ovariectomized  $ER\beta$ KO mice exhibit increased transcription of E2-sensitive genes and better cognition. Acute E2 treatment decreases transcription and fails to improve memory and chronic treatment impairs memory. (B) For  $ER\alpha$ KO mice, the ascending arm of the dose response function for memory and  $17\beta$ -estradiol (E2)- mediated transcription is shifted to the right. In this case, memory impairment is observed in low estrogen condition such as following ovariectomy and E2 treatment improves cognition and increases E2-sensitive transcription.



**Figure 5.** Post-translational regulation of estrogen receptor expression. Ligand-bound estrogen receptor-alpha ( $ER\alpha$ )- mediated transcription is coupled with the Hsc70-interacting protein (CHIP) and ubiquination–proteolysis process, such that ligand-bound  $ER\alpha$  is degraded following successful transcription of the target gene. In contrast,  $ER\beta$  interacts with CHIP and ubiquination and subsequent ligand binding can induce transcription or transport to the proteasome for degradation. Thus, ligand-bound receptor degradation may indicate ongoing transcription for  $ER\alpha$  or act to down-regulate  $ER\beta$  transcriptional activity. Other posttranscriptional modifications include  $ER\alpha$  miRNAs that bind to the 3' untranslated region and prevent mRNA stability or translation.



**Figure 6.** Transcriptional regulation of estrogen receptor expression. The promoter region of the estrogen receptor alpha (ER $\alpha$ ) gene (ESR1) contains CpG sites and methylation within this region is associated with reduced transcription of ESR1. Successful transcription requires histone acetyltransferase (HAT) enzyme activity to increase access to DNA for transcription. Histone deacetylases (HDACs) prevent the unwinding of the DNA from the histone thereby preventing transcription.