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Review

Long-term consequences of estrogens administered in midlife on female cognitive aging



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ABSTRACT

This article is part of a Special Issue "Estradiol and cognition".

Many of the biochemical, structural, and functional changes that occur as the female brain ages are influenced by changes in levels of estrogens. Administration of estrogens begun during a critical window near menopause is hypothesized to prevent or delay age-associated cognitive decline. However, due to potential health risks women often limit use of estrogen therapy to a few years to treat menopausal symptoms. The long-term consequences for the brain of short-term use of estrogens are unknown. Interestingly, there are preliminary data to suggest that short-term use of estrogens during the menopausal transition may afford long-term cognitive benefits to women as they age. Thus, there is the intriguing possibility that short-term estrogen therapy may provide lasting benefits to the brain and cognition. The focus of the current review is an examination of the long-term impact for cognition of midlife use of estrogens. We review data from our lab and others indicating that the ability of midlife estrogens to impact estrogen receptors in the hippocampus may contribute to its ability to exert lasting impacts on cognition in aging females.

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Results of research conducted over the last two decades support a role for estrogens in the modulation of cognitive function (reviewed in Boulware et al., 2012; Luine, 2014; Bimonte-Nelson et al., 2010). Many, although not all, randomized clinical trials and observational studies have reported that postmenopausal estrogen therapy is associated with improved cognition if treatment is initiated within a critical period after loss of ovarian function (Sherwin, 2009). However the potential health risks associated with exposure to estrogens (Chen and Colditz, 2007; Chen et al., 2006; but see Harman et al., 2011) may preclude their long-term use. Therefore, current recommendations include limiting the use of hormone therapy to a few years to treat menopausal symptoms. It is currently unknown if estrogen use for a few years in midlife will reduce risk of dementia or improve cognitive aging later in life. The current report provides an overview of the literature describing the long-term impact for cognition of midlife estradiol use. We also describe our recent work investigating mechanisms by which short-term estradiol administration in midlife can exert long-term benefits for memory.

Estrogens and cognitive aging

Effects of estrogens on cognition in women

At menopause, circulating levels of estradiol, the main estrogen produced by the ovaries, drops to one-tenth of those during menstruating years (Rannevik et al., 2008). This dramatic change in hormonal state is proposed to have functional consequences for cognition (Sherwin, 1998), either directly or by interaction with other normal or pathological aging-related physiological alterations. In support of this hypothesis, many, though not all, randomized clinical trials and observational studies have reported a link between estrogen therapy initiated after naturally occurring or surgically-induced menopause in healthy women and improved cognition (reviewed in Sherwin, 2002). Findings of early randomized clinical trials that estrogen therapy positively influenced cognition suggested a possible protective role of estrogens against Alzheimer's disease. Supporting evidence was provided by many (Fillit et al., 1986; Honjo et al., 1995; Kawas et al., 1997; Ohkura et al., 1994; Paganini-Hill and Henderson, 1996; Tang et al., 1996), but not all (Brenner et al., 1994; Mulnard et al., 2000) studies demonstrating estrogen therapy was associated with reduced risk and severity, and delayed onset of Alzheimer's disease.

In order to systematically and fully evaluate the efficacy of hormone therapy, the National Institutes of Health established the Women's Health Initiative (WHI), a longitudinal study initiated in the 1990s that was designed to assess the efficacy of hormone therapy on the incidence, prevalence, and severity of cardiovascular disease, cancer, and osteoporosis in postmenopausal women. The objective of the auxiliary Women's Health Initiative Memory Study (WHIMS) was to determine the effect of postmenopausal hormone therapy on the development and progression of dementia and global cognitive function. Surprisingly, the results of the WHI and WHIMS indicated that hormone therapy regimens consisting of chronic conjugated equine estrogens (CEE) or CEE plus medroxyprogesterone as compared to placebo treatment, had no effect, or under certain conditions increased the risks of cardiovascular disease, breast cancer, stroke, dementia, and global cognitive decline (Chlebowski et al., 2003; Craig et al., 2005; Espeland et al., 2004; Rapp et al., 2003b; Rossouw et al., 2002; Shumaker et al., 2003, 2004; Wassertheil-Smoller et al., 2003). Scrutiny of the WHIMS design, population, specifics of hormone therapy regimen used, and tests of cognitive functioning has led to hypotheses that the failure of the WHIMS to demonstrate the predicted beneficial effects of hormone therapy may be explained by various confounding factors such as the advanced age and health problems of the participants, treatment specifics (agent, regimen, dose, and route of administration), and years of ovarian hormone deprivation the participants had already experienced (Harman et al., 2005).

Critical period hypothesis of effects of estrogens on cognition

In the WHIMS, the average age of the participants at the beginning of hormone treatment was 69 (Coker et al., 2009). Importantly, these women had been without ovarian hormones for nearly two decades. The "critical period" hypothesis of hormone therapy states that there is a crucial window following the onset of menopause during which hormone therapy must be initiated in order to have beneficial effects (Gibbs and Gabor, 2003; Resnick and Henderson, 2002). The brain may lose its responsiveness to estrogens after a prolonged absence of the steroids and estrogen sensitivity may remain only with a timely onset of hormone therapy. Furthermore, once brain structures have been without estrogens for too long, hormone therapy may have detrimental effects (Brinton, 2005; Suzuki et al., 2007). Therefore, the negative outcomes seen in the WHIMS may be due to the timing of hormone therapy initiation. A recent review summarizes the clinical literature including observational studies and small randomized clinical trials examining the impact of early initiation of hormone therapy on cognitive outcomes and concludes that there exists initial support for the critical period hypothesis (Maki, 2013). Results of ongoing or recently completed randomized, placebo-controlled clinical trials should provide additional insights (Wharton et al., 2013; Hodis et al., 2015).

Experimental tests of the critical period hypothesis using animal models provide corroborating evidence. For instance, nonhuman primate studies have reported that estrogens administered 30 weeks, but not 10 years post ovariectomy improves performance on tests of working memory (Lacreuse et al., 2002; Rapp et al., 2003a). In rodent models, Gibbs (2000b) first showed that long-term hormone deprivation prevented the ability of later estradiol administration to enhance hippocampus-dependent memory in aging female rats. Middle-aged rats were ovariectomized and chronically treated with estradiol beginning either immediately, 3 months, or 10 months later. Only rats treated immediately or 3 months post-ovariectomy showed enhanced performance on a delayed matching-to-position maze task when tested in old age. Similarly, our lab demonstrated that the length of hormone deprivation impacts the ability of exogenous estradiol treatment to enhance hippocampus-dependent memory in aging female rats (Daniel et al., 2006). Rats ovariectomized at either 12 or 17 months of age and immediately implanted with estradiol capsules outperformed ovariectomized controls when tested at 17.5 months of age. However, rats ovariectomized at 12 months of age and implanted with estradiol capsules at 17 months did not show enhanced memory compared to controls. Therefore, both a short-term (2 weeks) and long-term (5 months) estradiol regimen was effective at enhancing memory when initiated immediately following ovariectomy, but estradiol treatment initiated 5 months post-ovariectomy was ineffective. Furthermore, a related study in our lab showed that this effect is not limited to hippocampus-dependent tasks (Bohacek and Daniel, 2010). Middle-aged rats that were immediately implanted with estradiol capsules following ovariectomy displayed enhanced performance on the 5-choice serial reaction time task, an attentional task dependent upon the prefrontal cortex, compared to ovariectomized control rats. However, estradiol treatment initiated 5 months post-ovariectomy did not enhance performance on the task compared to controls. Collectively, these results support the idea that there is a critical time window following the cessation of ovarian function during which estradiol administration must be initiated in order to exert positive effects on cognition.

Long-term effects on cognition of short-term estrogen use within the critical period

The critical period encompassing the menopausal transition, during which evidence suggests estrogen therapy must be initiated in order to benefit cognition, is reminiscent of the hormone-sensitive period in early development during which hormones act on the brain to exert permanent influences on adult behavioral responsiveness to hormones

(Phoenix et al., 1959). Traditionally, effects of gonadal hormones on the brain and behavior have been categorized as organizational or activational. Organizational effects occur during early developmental periods, are long-lasting, and persist beyond the period of exposure to the hormone. Activational effects occur in the adult, are generally temporary, and are expressed only in the presence of the hormone. However, it has become apparent that effects of hormones do not always fit this conceptual dichotomy (Schulz et al., 2009). As early as several decades ago, it was proposed that there are multiple sensitive periods during which the nervous system could be permanently altered and that these sensitive periods most likely occur during times of rapid change (Scott et al., 1974). In support of this proposal are data indicating that ovarian hormones during the adolescent period exert lasting impact on adult behaviors including patterns of food protection (Field et al., 2004) and ingestive (Swithers et al., 2008) behaviors. Like the adolescent period, middle-age in females is characterized by significant changes in levels of ovarian hormones and thus may represent another sensitive period during which the nervous system could be permanently altered. Whereas current evidence supporting the idea of a critical period during midlife indicates that the absence of exogenously administered estrogens following menopause permanently alters the system, it may also be the case that the presence of exogenously administered estrogens could permanently alter the system. If so, this raises the

possibility that hormone exposure during the menopausal transition could have consequences for the brain and behavior long after the duration of exposure.

There is a small literature available to suggest that short-term exposure to estrogens during the critical period after the loss of ovarian function may provide long-lasting cognitive benefits for women. For example, the risk for cognitive impairment in women who received 2–3 years of hormone therapy in the early menopausal years as part of a randomized, placebo-controlled study was decreased by 64% when they were examined 5–15 years after the completion of the hormone therapy (Bagger et al., 2005). In addition, risks of all-cause mortality, cardiovascular mortality, coronary heart disease, and osteoporotic fracture were also reduced in these women (Alexandersen et al., 2006; Bagger et al., 2004). Results of a large population-based study indicated that compared to never users, women using hormone therapy only in midlife had a 26% reduced risk of developing dementia later in life (Whitmer et al., 2011). In contrast, women using hormone therapy only in late life had a 48% increased risk of dementia and hormone therapy used in both mid and late life had no effect on dementia risk. Emerging evidence indicates that short-term use of estrogens following surgical menopause induced by bilateral oophorectomy before the age of natural menopause provides long-term neuroprotection (for review, see Rocca et al., 2014). For example, long-term risk of cognitive

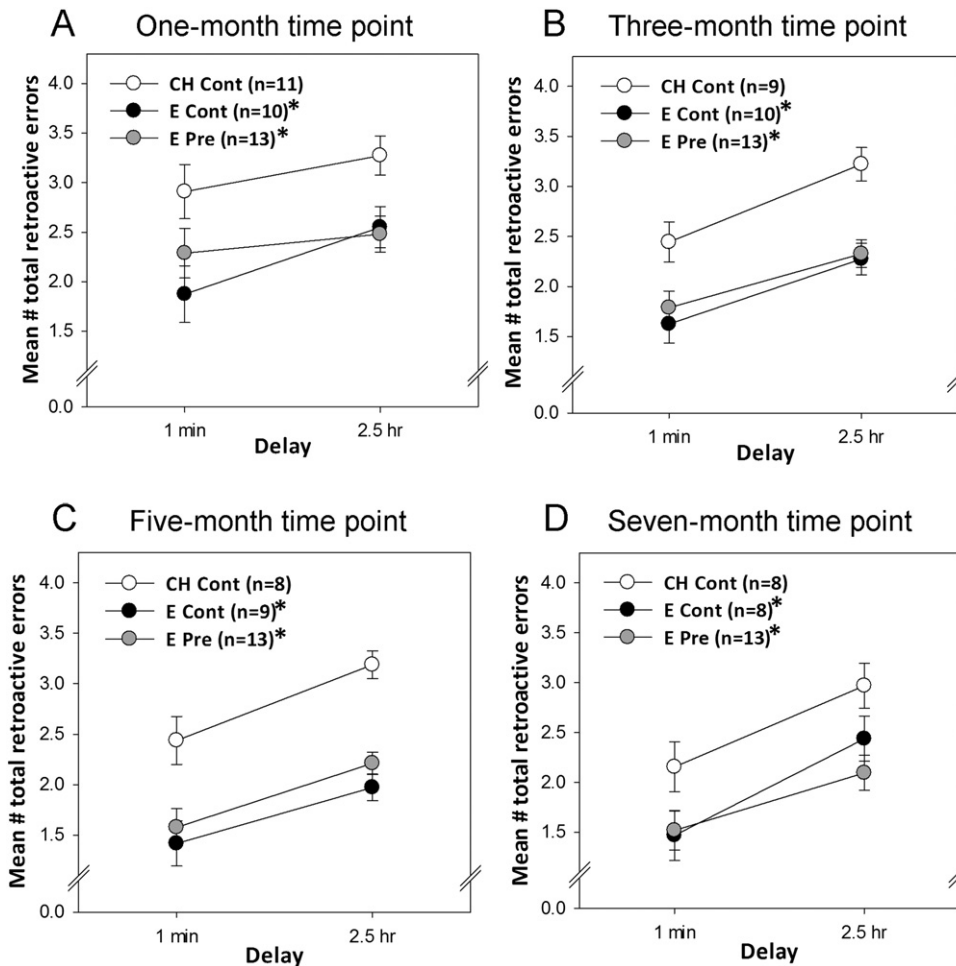


Fig. 1. Impact of ongoing and previous exposure to estradiol on memory in aging female rats. Long-Evans rats (retired breeders) were ovariectomized in middle age (at ~11 months of age) and received subcutaneous implants of capsules containing estradiol or cholesterol vehicle. Capsules remained in place for 40 days. After 40 days, half of the rats with estradiol implants received new estradiol implants resulting in continuous estradiol treatment previous to and throughout behavior testing (E Cont). The other half received cholesterol implants resulting in exposure to estradiol previous to behavior testing and control treatment throughout testing (E Pre). Rats that had cholesterol capsules received new cholesterol implants resulting in continuous cholesterol treatment previous to and throughout testing (Ch Cont). Rats then were tested every two months on delay trials in a radial-arm maze task in which delays of 1 min or 2.5 h were imposed between the 4th and 5th arm choices. Mean number of total retroactive errors (\pm SEM) during 1 min and 2.5 h delay trials averaged over 4-trial blocks one (A), three (B), five (C), and seven (D) months after termination of estradiol treatment in the E Pre group. * $p < .05$ vs. CH Cont (Rodgers et al., 2010).

impairment was almost doubled in women who underwent oophorectomy before menopause, but was eliminated in women undergoing estrogen therapy until at least the age of 50 (Rocca et al., 2007). Additionally, the detrimental effects of early surgical menopause on cognition were attenuated by hormone therapy initiated within five years of surgery and lasting at least ten years (Bove et al., 2014). However, not all reports indicate long-term effects of midlife estrogen use on later cognitive function (Hogervorst and Bandelow, 2010). For example, an average of seven years of hormone therapy initiated when women were 50–55 years of age had no beneficial or detrimental effects as compared to placebo treatment on cognitive function assessed seven years after the cessation of the hormone therapy (Espeland et al., 2013). Although further work needs to be done, collectively these data indicate at least the possibility that under some conditions estrogen therapy initiated during a critical window following natural or surgical menopause can provide long-term cognitive benefits that persist beyond the period of exposure.

In our lab, we sought to test experimentally the hypothesis that short-term exogenously administered estrogens in midlife can permanently organize the system to provide lasting benefits to hippocampus-dependent memory in aging female rats. We compared the ability of previous estradiol administration and ongoing continuous estradiol administration to impact spatial memory in a radial-arm maze (Rodgers et al., 2010). Middle-aged rats were ovariectomized and received control treatment, continuous estradiol treatment via Silastic implants, or 40 days of estradiol treatment that was terminated prior to behavior testing. Circulating estradiol levels produced by our capsules are between 26 and 47 pg/ml, a low to mid physiological range (Bohacek and Daniel, 2010). We chose 40 days for our short-term treatment paradigm because based on average life expectancy of 2.5 years for Long–Evans rats (Harlan Sprague–Dawley, Inc.) and of 80.1 years for women (Hamilton et al., 2007), a 40-day period of estradiol exposure in rats would roughly correspond to 3.5 years of estrogen therapy in women. Spatial memory was assessed every two months beginning one month after the termination of the 40-day estradiol period. As illustrated in Fig. 1, previous exposure to estradiol was just as effective as ongoing use in enhancing performance. The effect of the previous estradiol exposure was maintained for up to seven months after the exposure had been terminated. Thus, these experimental data provide support for the hypothesis that short-term exposure to estrogens in midlife provides lasting cognitive benefits that persist well beyond the period of exposure. The neural mechanisms by which these cognitive benefits are mediated are under investigation. However, because of the growing evidence indicating the importance of estrogen receptors in female cognitive aging (Daniel, 2013; Foster, 2012), we explored a role for estrogen receptors in the ability of short-term estradiol exposure to exert long-term impact on memory.

The importance of estrogen receptors in brain and cognitive aging

Estrogens act at estrogen receptors to exert effects

Estrogens act at estrogen receptors (ER) through both traditional genomic mechanisms as well as via nontraditional rapid effects at the membrane. In the traditional model of estrogen action, estrogen binds to either ER α or ER β in the nucleus, allowing it to dimerize and bind to a DNA estrogen response element (ERE) or interact with a transcription factor on target genes, thus initiating transcription of estrogen-sensitive genes and proteins, a process on the time scale of hours (Cowley et al., 1997; Gaub et al., 1990). ER α and ER β share a high degree of homology; however they only overlap by about 60% in their sequence for the ligand-binding region (Tremblay et al., 1997). This difference in the ligand-binding region may explain the higher affinity that estradiol has for ER α over ER β and the ability of ER α to more successfully induce transcription linked to the ERE.

Estrogens can also have rapid effects not dependent on traditional genomic mechanisms (Fernandez et al., 2008). In this non-classical mechanism, estrogens bind to membrane-bound receptors, including the G-protein coupled estrogen receptor (GPER), which can then activate second messenger systems, causing a rapid response varying from seconds to minutes (Prossnitz et al., 2008). Whereas nuclear ER α or ER β activation result in a traditional genomic response (McEwen and Alves, 1999), it has been proposed that these receptors or a modified form of the proteins (Acconcia et al., 2005; Li et al., 2003) also contribute to the rapid effects of estradiol on synaptic plasticity (Ooishi et al., 2012). While the cellular and subcellular sites of ER α in the hippocampus have not been completely elucidated, it has been localized to both nuclear and extranuclear sites (Spencer et al., 2008), including in spines of hippocampal pyramidal and granule neurons (Mukai et al., 2007) as well as in axon terminals of cholinergic neurons (Towart et al., 2003). The genomic and non-genomic effects of estradiol are not mutually exclusive, as intracellular cascades activated by estrogens can phosphorylate nuclear estrogen receptors (Lannigan, 2003), allowing for translocation to the nucleus (Lee and Bai, 2002), dimerization (Chen et al., 1999) and transcriptional activity (Ali et al., 1993; Le et al., 1994). Evidence suggests a combination of both membrane-initiated and genomic actions occurring either in parallel or sequentially at estrogen receptors to affect transcription (Bjornstrom and Sjoberg, 2005; Vasudevan and Pfaff, 2008).

Estrogen receptors and the critical period hypothesis

There is increasing evidence that changes in levels or responsiveness of estrogen receptors, particularly ER α , may explain the existence of a “critical period” following the cessation of ovarian function during which estrogens must be administered to exert effects on the brain and cognition (Daniel, 2013). ER α , perhaps due to its increased ability to induce transcription linked to the ERE as compared to ER β (Tremblay et al., 1997), is particularly important for maintaining hippocampal function under low estradiol levels (Foster, 2012). It has been suggested that a decrease in estradiol responsiveness in the hippocampus following the loss of ovarian function may be due to age-related decline in levels of ER α , disrupting the ratio of ER α relative to ER β and interfering with the transcriptional processes important for cognition (Bean et al., 2014; Foster, 2012). Furthermore, results of work from our lab demonstrated that the same regimen of ongoing estradiol treatment that results in enhancement of hippocampus-dependent memory results in increased levels of ER α in the hippocampus. Specifically, estradiol administration initiated at the time of ovariectomy, but not after long-term ovarian hormone deprivation enhances spatial memory (Daniel et al., 2006) and increases levels of ER α in the hippocampus (Bohacek and Daniel, 2009). No impact on levels of ER β was evident.

Interestingly, evidence for a mechanistic explanation for a critical period has emerged that involves the necessity of maintaining a sufficient pool of ER α in the hippocampus in order for the neuroprotective effects of estradiol to be maintained following cessation of ovarian function (Zhang et al., 2011). Ten weeks following ovariectomy, ER α in rat hippocampus displayed enhanced interaction with the E3 ubiquitin ligase C terminus of heat shock cognate protein 70 (Hsc70)-interacting protein (CHIP) that leads to its ubiquitination/proteasomal degradation. Estradiol treatment initiated at the time of ovariectomy, but not ten weeks following ovariectomy, prevented that enhanced ER α -CHIP interaction and the ER α ubiquitination/proteasomal degradation. Furthermore, estradiol treatment initiated at the time of ovariectomy, but not ten weeks following ovariectomy was fully neuroprotective against global cerebral ischemia. Thus, the critical period may exist because of permanent alterations in levels of ER α that occur following long-term hormone deprivation.

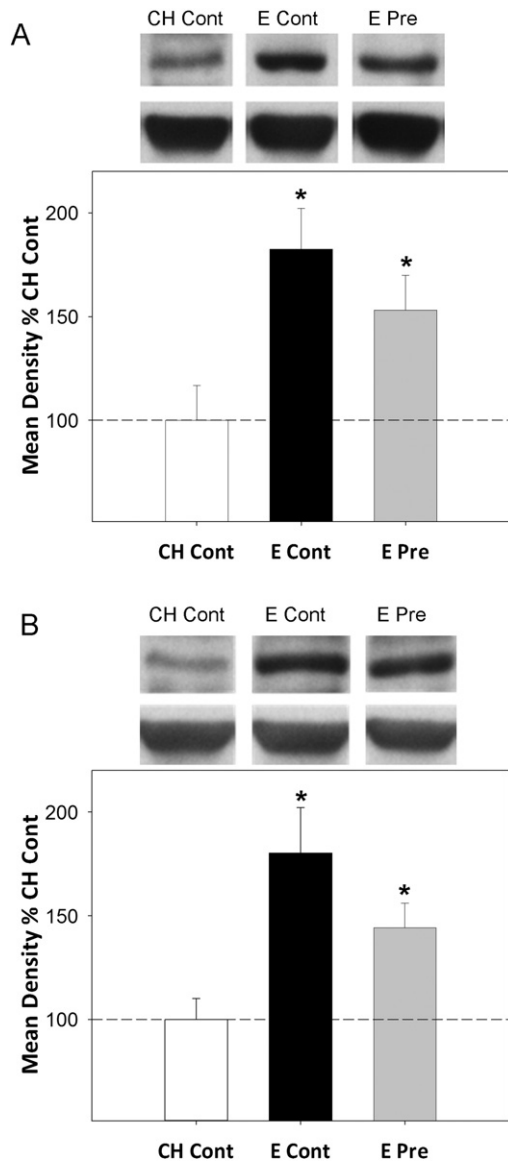


Fig. 2. Impact of ongoing and previous exposure to estradiol on levels of ER α protein in the hippocampus of aging female rats as measured by western blotting. Long-Evans rats (retired breeders; $n = 8-13$ per group) were ovariectomized in middle age (at ~ 11 months of age) and received subcutaneous implants of capsules containing estradiol or cholesterol vehicle. Capsules remained in place for 40 days. After 40 days, half of the rats with estradiol implants received new estradiol implants resulting in continuous estradiol until sacrifice (E Cont). The other half received cholesterol implants resulting in 40 days of previous exposure to midlife estradiol followed by control treatment until sacrifice (E Pre). Rats that had cholesterol capsules received new cholesterol implants resulting in continuous cholesterol control treatment until sacrifice (Ch Cont). Western blot data are from brains collected from behaviorally tested rats sacrificed three months (A) and eight months (B) after termination of estradiol treatment in the E Pre group. Mean optical density (+SEM) expressed relative to CH Cont. * $p < .05$ vs. CH Cont. Representative blot images for ER α and the loading control, β -actin are shown in inserts above respective graphs (Rodgers et al., 2010).

Long-term impact of short-term estrogens on levels of estrogen receptor in the hippocampus

Because long-term estradiol deprivation during the critical period resulted in a permanent decrease in levels of hippocampal ER α (Bohacek and Daniel, 2009), we determined if there was also a long-term impact of the presence of estradiol during the critical period on levels of ER α in the hippocampus. We investigated if the same short-term exposure to estradiol that results in lasting enhancement of hippocampus-dependent memory (Fig. 1) would also result in lasting increases in levels of ER α in

the hippocampus (Rodgers et al., 2010). Continuous estradiol treatment as well as 40 days of estradiol exposure that was terminated \sim three months prior to sacrifice significantly increased ER α protein levels in the hippocampus of ovariectomized rats as compared to ovariectomized controls as measured by western blotting (See Fig. 2A). There were no effects of treatments on ER β levels (data not shown). Results of a second experiment in which ovariectomized rats were sacrificed \sim 8 months after the 40-day prior estradiol treatment was terminated revealed continued elevation of ER α in the hippocampus as compared to control rats that were never exposed to estradiol (See Fig. 2B). Interestingly, prior estradiol treatment also increased levels of choline acetyltransferase (ChAT), the synthesizing enzyme for acetylcholine, for up to three months following termination of exposure (Rodgers et al., 2010). Acetylcholine is a neurotransmitter implicated in the regulation of learning and memory (Everitt and Robbins, 1997) and the ability of the estradiol to impact the cholinergic system (Luine, 1985; Gabor et al., 2003; Gibbs, 2000a) is related to its ability to impact hippocampus-dependent memory (Daniel and Dohanich, 2001). ER α -mediated regulation of ChAT occurs at the transcriptional level and the identification of a putative estrogen response element on the ChAT gene provides a potential site for direct modulation of ChAT by ER α (Hyder et al., 1999; Miller et al., 1999). Thus, a short-term period of 40 days of estradiol exposure can provide both lasting memory enhancements and lasting increases in levels of ER α and ER α -regulated proteins in the hippocampus, long after the estradiol exposure is terminated. A causal relationship between the ability of previous estradiol to

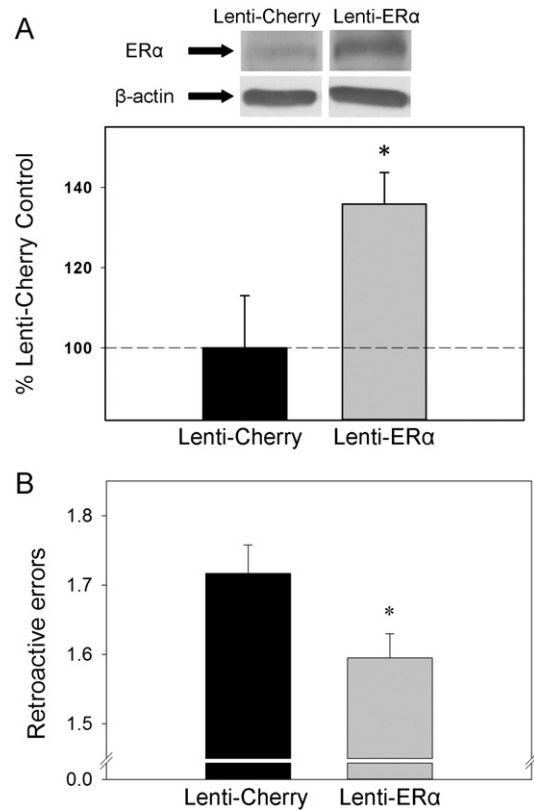


Fig. 3. Impact of increasing levels of ER α in the hippocampus on memory in ovariectomized aging female rats. Long-Evans rats (retired breeders; $n = 5-7$ per group) were ovariectomized in middle age (at ~ 11 months of age). Forty days following ovariectomies, rats received intrahippocampal infusions of either a lentivirus with the gene encoding ER α (Lenti-ER α) or a control virus (Lenti-Cherry). Beginning three weeks following virus infusions, spatial memory performance was assessed in a radial-arm maze with various delays imposed between the 4th and 5th arm choices. (A) Protein expression of ER α in the hippocampus as measured by western blotting in brains collected following completion of behavior testing. Mean density x area (+SEM) expressed relative to Lenti-Cherry control. Representative blot images for ER α and the loading control β -actin are shown in insets above the graph. (B) Mean number of retroactive errors (+SEM) averaged across delays. * $p < .05$ vs. Lenti-Cherry (Witty et al., 2012).

impact ER α levels and impact memory is suggested by mounting evidence indicating that increases in the expression of ER α in the hippocampus may preserve cognitive function in the aging brain in the absence of ovarian estrogens (Han et al., 2013; Witty et al., 2012).

Estrogen receptors impact cognition in the absence of ovarian or exogenously administered estrogens

The importance of ER α to cognitive aging is highlighted by results of studies examining the relationship between levels of wild-type and polymorphisms of ER α with development of cognitive impairment in women. For example, a significant positive relationship was found between scores on the Mini-Mental State Examination (MMSE) and levels of wild-type ER α in the prefrontal cortex of Alzheimer's patients (Kelly et al., 2008). No relationship was apparent between MMSE scores and levels of ER β . Furthermore, several (Brandi et al., 1999; Isoe-Wada et al., 1999; Mattila et al., 2000), but not all (Maruyama et al., 2000) studies report that levels of ER α gene polymorphisms are associated with an increased risk for Alzheimer's disease. Finally, in non-demented community-dwelling women (Yaffe et al., 2002, 2009) and men (Yaffe et al., 2009), polymorphisms of ER α are associated with increased risk of age-related cognitive decline.

Foster et al. (2008) used an ER α knockout (ER α KO) mouse model to directly test the importance of ER α on hippocampus-dependent spatial memory. Ovariectomized, young adult ER α KO mice exhibited learning and memory deficits compared to their ovariectomized wild-type littermates. Injection of a lentivirus delivering the gene expressing ER α to the hippocampus of ovariectomized ER α KO mice significantly improved cognitive performance. Importantly, the effects on cognition were apparent in the absence of exogenously administered estrogens, highlighting the importance of ER α to cognition under conditions of low circulating estrogens.

In our lab, we investigated if the level of ER α in the hippocampus of aging females would have an impact on memory in the absence of ovarian or exogenously administered estrogens (Witty et al., 2012). We delivered a lentivirus encoding the gene for ER α to the hippocampus of middle-aged rats that had been ovariectomized 40 days prior to the delivery. Our procedure resulted in an approximately 40% increase in levels of ER α protein as measured by western blotting (see Fig. 3A). We tested rats on a hippocampal dependent radial-maze task in which rats have to remember the location of food rewards across varying time delays. As illustrated in Fig. 3B, increasing ER α levels in the hippocampus resulted in improved memory in aging females in the absence of ovarian or exogenously administered estrogens. These data provide support for the hypothesis that those treatments that increase or maintain levels of ER α in aging females help to improve or maintain memory processes even in the absence of circulating estrogens. Furthermore, they are consistent with the idea that the ability of short-term midlife estradiol treatment to provide lasting increases in levels of ER α in the hippocampus in aging females (Fig. 2) underlies its ability to provide lasting memory enhancements (Fig. 1).

Mechanisms by which estrogen receptor can impact cognition in the absence of ovarian or exogenous estrogens

Ligand-independent mechanisms involving insulin-like growth factor-1

In addition to hormone-mediated estrogen receptor action, estrogen receptor function can be modulated by extracellular signals (Smith, 1998), which provides a mechanism by which estrogen receptor can impact target tissue under conditions of low levels of endogenous estrogens. These ligand-independent actions include the ability of growth factors, such as insulin-like growth factor-1 (IGF-1), to activate estrogen receptor and increase expression of estrogen receptor target genes. In addition to its endocrine role, IGF-1 is produced and acts locally in many tissues, including brain (LeRoith, 2008). IGF-1 exerts its effects

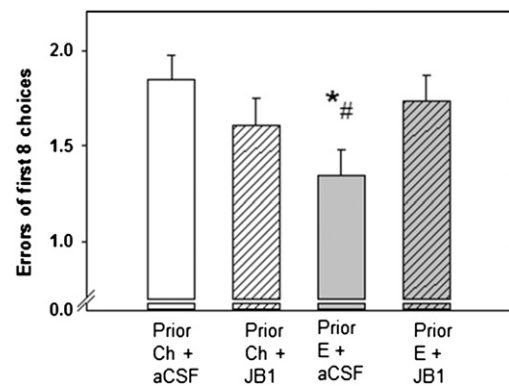


Fig. 4. Effects of previous treatment with estradiol and subsequent antagonism of brain insulin-like growth factor-1 (IGF-1) receptors on memory in aging ovariectomized rats. Long-Evans rats (retired breeders; $n = 8-10$ per group) were ovariectomized in middle age (at ~11 months of age) and implanted with capsules containing estradiol (Prior E) or cholesterol vehicle (Prior Ch). After 40 days all capsules were removed. Chronic icv delivery via cannulae attached to osmotic minipumps of the IGF-1 receptor antagonist, JB1, or aCSF vehicle was initiated and continued for approximately 30 days. Spatial memory performance was assessed in a radial-arm maze with various delays imposed between the 4th and 5th arm choices. Data represent mean number of errors of first eight arm choices (\pm SEM) averaged over all delays. * $p < .05$ vs. Prior Ch + aCSF; # $p < .05$ Prior E + JB1 (Witty et al., 2013).

by binding to IGF-1 receptor, which is a transmembrane protein with tyrosine kinase activity. Particularly relevant for memory is evidence of high level of colocalization of ER α and IGF-1 receptors in CA1 of the hippocampus (Cardona-Gomez et al., 2000).

It has been demonstrated in a number of different cell lines, including neuroblastoma cells, that in the absence of estrogens, IGF-1 activates ER α and regulates ER α -mediated gene transcription (Agrati et al., 1997; Font de and Brown, 2000; Martin et al., 2000; Stoica et al., 2000; Mendez and Garcia-Segura, 2006; Klotz et al., 2002). The mechanism by which this occurs is unclear, but most likely involves modification of phosphorylation sites on ER α by cellular kinases (Hall et al., 2001). Activation of IGF-1 receptors leads to activation of two main downstream signaling cascades, the Ras-ERK/MAPK and PI3K-Akt pathways (Russo et al., 2005), both of which are involved in regulation of ER α transcription (Martin et al., 2000; Mendez and Garcia-Segura, 2006; Patrone et al., 1998; Kato et al., 1995). Interestingly, growth factor phosphorylation of ER α occurs via the ERK/MAPK pathway at Ser118 (Kato et al., 1995) and ER α phosphorylated at Ser 118 is resistant to proteasomal degradation (Valley et al., 2008). Thus, there are putative mechanisms involving growth factor signaling by which ER α levels may be maintained beyond the period of estradiol exposure.

We hypothesized that the increased levels of ER α present in aging females that have been exposed to prior short-term estradiol (Fig. 1) would allow for increased activation of hippocampal ER α by a ligand-independent mechanism involving IGF-1. This increased activation would result in increased expression of ER α target genes and associated proteins and ultimately improved performance on hippocampus-dependent tasks. As an initial test of this hypothesis, we investigated the role of IGF-1 receptors in the ability of short-term prior exposure to estradiol to exert lasting effects on cognition (Witty et al., 2013). Middle-age rats were ovariectomized and treated with vehicle or estradiol delivered via Silastic capsules. After 40 days, all capsules were removed and rats were implanted with cannulae (icv) attached to osmotic minipumps that delivered vehicle or the IGF-1 receptor antagonist, JB1. Rats were tested on trials in the radial-arm maze during which delays were imposed between the 4th and 5th arm choices. As illustrated in Fig. 4, administration of JB1 blocked the ability of prior exposure to estradiol to enhance performance without significantly affecting performance in the ovariectomized controls. Furthermore, JB1 blocked the ability of previous exposure to estradiol to result in lasting increases in levels of ER α and the ER α -regulated protein, ChAT (see

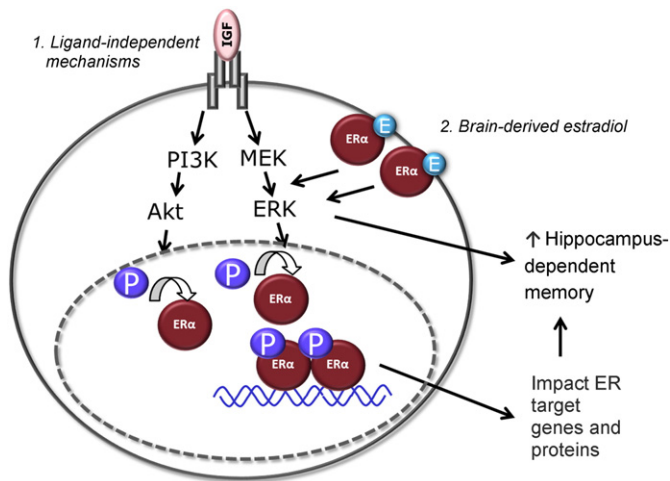


Fig. 5. Hypothesized model involving actions at ER α by which previous exposure to estradiol can influence memory beyond the period of estradiol exposure. Midlife estradiol increases levels of ER α in the hippocampus. Levels are maintained beyond the period of estradiol exposure. This increased pool of ER α allows for: (1) Insulin-like growth factor-1 (IGF-1) acting at its receptor activates intracellular signaling cascades. Action involving one or both of these signaling cascades culminates in phosphorylation (P) and activation of ER α at estrogen response element (ERE)-containing promoters. This allows for increased ER α -mediated transcription affecting levels of ER α -regulated target genes and proteins in the hippocampus resulting in enhancement in hippocampus-dependent memory. (2) Hippocampus-derived estradiol (E) activates membrane-associated ER α receptors, which activates intracellular signaling cascades. This activation can have rapid effects on memory and can also culminate in phosphorylation and activation of nuclear ER α .

Witty et al., 2013). Results indicate that activation of IGF-1 receptors is a necessary component in the ability of prior estradiol exposure to exert lasting benefits to cognition. Importantly, they also demonstrate that the decrease in performance induced by antagonism of IGF-1 receptors is specific to rats that had prior treatment with estradiol and not due to a generalized impairment induced by JB1 on cognition. Though further work is needed to confirm a direct interaction between IGF-1 and ER α , these results are consistent with the hypothesis that the lasting increase in levels of ER α resulting from previous estradiol exposure impacts memory via mechanism involving IGF-1 signaling.

Local estradiol synthesis

In addition to putative ligand-independent mechanisms, in the absence of ovarian hormones or estradiol administration ER α may be activated by locally synthesized estrogens in the brain. All the proteins necessary for estradiol synthesis are expressed in the hippocampus (Compagnone and Mellon, 2000; Wehrenberg et al., 2001). Adult hippocampal neurons synthesize estradiol *in vitro*, an effect that can be attenuated by inhibition of aromatase, the enzyme that initiates the final step in estradiol synthesis (Kretz et al., 2004). Hippocampus-derived estradiol may be particularly important for rapid activation of estrogen receptor at the membrane, including membrane associated ER α (Ishii et al., 2007). These rapid actions include the ability of ER α induce activation of the ERK/MAPK pathway. Consistent with an effect of ER α -induced activation of the ERK/MAPK pathway are data from our lab demonstrating that increasing levels of hippocampus ER α to ovariectomized rats via lentiviral delivery results in increased activation of the ERK/MAPK pathway (Witty et al., 2012). Furthermore, increased levels of ER α resulting from previous exposure to estradiol were also associated with increased activation of the ERK/MAPK pathway (Witty et al., 2013). Highlighting the importance of extragonadal estrogens, likely including brain estradiol, in memory are data indicating that post-menopausal women treated for breast cancer with aromatase inhibitors exhibit memory deficits (Shilling et al., 2003). Additionally, ovariectomized female aromatase knockout mice have impaired memory as compared to ovariectomized wild-types (Martin et al., 2003). Thus, brain-

derived estradiol may provide a mechanism by which ER α signaling can impact memory following the cessation of ovarian function.

Hypothesized model by which midlife estradiol can exert lasting impact on memory

Fig. 5 depicts a hypothesized model by which we propose that midlife estradiol can exert long-term impacts on memory beyond the period of estradiol exposure. Previous estradiol exposure allows for lasting increases in levels of ER α in the hippocampus (see Fig. 2 and Rodgers et al., 2010; Witty et al., 2013). These increases in ER α would allow for novel mechanisms of activation in the absence of circulating estrogens. First, increased ER α levels would allow for increased effects resulting from ligand-independent activation of estrogen receptor by which growth-factors, including IGF-1, could initiate ER α -dependent transcription, increasing levels of ER α -target proteins, leading to enhanced memory. Our data, indicating that antagonism of IGF-1 receptors blocks the effects of previous estradiol on ER α -dependent proteins and on memory (Witty et al., 2013) are consistent with this hypothesized mechanism. Second, increased levels of ER α associated with the membrane would allow for rapid synaptic effects via actions of locally synthesized estradiol in the hippocampus that could exert rapid effects on memory and also impact transcription via intracellular signaling pathways. Although further work is needed to directly test this hypothesized mechanism, our data showing that increased ER α levels in the hippocampus are associated with increased ERK/MAPK signaling (Witty et al., 2012, 2013) are consistent with a role for ER α in rapid intracellular signaling in the absence of ovarian estrogens.

Conclusions

The long-term impact for the brain and cognition of short-term use of hormone therapy in middle-age such as that used by women during the menopausal transition is currently unclear. However, preliminary data in the clinical literature are suggestive of long-term neuroprotective effects of short-term administration of estrogens within a critical time window following natural or surgical menopause. Consistent with such an effect are data from our lab that demonstrate that in a rat model of menopause, 40 days of estradiol administration following ovariectomy provides long-lasting benefits to memory. Associated with these enhancements are increased levels of ER α in the hippocampus. We hypothesize that this increased pool of ER α in the hippocampus allows for novel mechanisms of activation in the absence of circulating estrogens that leads to improved memory. Many questions remain to be answered including the cellular localization of the increased pool of ER α resulting from previous estradiol exposure as well as the mechanisms by which estrogen receptor levels are maintained following the termination of estradiol treatment. Nevertheless, data across the clinical and basic science literature increasingly support a relationship between levels and/or functional status of brain ER α and cognitive aging. These data support the idea that treatments that can impact levels of estrogen receptor, even in the absence of circulating estrogens, can impact memory.

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