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Interactive Effects of Age and Estrogen on Cortical Neurons: Implications for Cognitive Aging

Megan Bailey[a], **Athena C.J. Wang**[a], **Jiandong Hao**[a,b], **William G.M. Janssen**[a], **Yuko Hara**[a], **Dani Dumitriu**[a], **Patrick R. Hof**[a], and **John H. Morrison**[a,*]

[a] Department of Neuroscience, Mount Sinai School of Medicine, New York, New York 10029

[b] Trauma Center, Third Hospital of Hebei Medical University, Hebei, China 050051

Abstract

In the past few decades it has become clear that estrogen signaling plays a much larger role in modulating the cognitive centers of the brain than previously thought possible. We have developed a nonhuman primate (NHP) model to investigate the relationships between estradiol (E) and cognitive aging. Our studies of cyclical E treatment in ovariectomized (OVX) young and aged rhesus monkeys have revealed compelling cognitive and synaptic effects of E in the context of aging. Delayed response (DR), a task that is particularly dependent on integrity of dorsolateral prefrontal cortex (dlPFC) area 46 revealed the following: 1) that young OVX rhesus monkeys perform equally well whether treated with E or vehicle (V), and 2) that aged OVX animals given E perform as well as young adults with or without E, whereas OVX V-treated aged animals display significant DR impairment. We have analyzed the structure of layer III pyramidal cells in area 46 in these same monkeys. We found both age and treatment effects on these neurons that are consistent with behavioral data. Briefly, reconstructions of pyramidal neurons in area 46 from these monkeys showed that cyclical E increased the density of small, thin spines in both young and aged monkeys. However, this effect of E was against a background of age-related loss of small, thin spines, leaving aged V-treated monkeys with a particularly low density of these highly plastic spines and vulnerable to cognitive decline. Our current interpretation is that E not only plays a critically important role in maintaining spine number, but also enables synaptic plasticity through a cyclical increase in small highly plastic spines that may be stabilized in the context of learning. Interestingly, recent studies demonstrate that chronic E is less effective at inducing spinogenesis than cyclical E. We have begun to link certain molecular attributes of excitatory synapses in area 46 to E effects and cognitive performance in these monkeys. Given the importance of synaptic estrogen receptor α (ER-α) in rat hippocampus, we focused our initial studies on synaptic ER-α in area 46. Three key findings have emerged from these studies: 1) synaptic ER-α is present in axospinous synapses in area 46; 2) it is stable across treatment and age groups (which is not the case in rat hippocampus); and 3) the abundance and distribution of synaptic ER-α is a key correlate of individual variation in cognitive performance in certain age and treatment groups. These findings have important implications for the design of hormone treatment strategies for both surgically and naturally menopausal women.

[*]To whom correspondence should be addressed. Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1065, New York, NY 10029, Tel: (212) 659-5985, Fax: (212) 849-2510, John.Morrison@mssm.edu.

Keywords

Prefrontal cortex; estrogen; aging; primate; cognition; hormone replacement therapy

1. Introduction

This year, the first members of the “baby boomer” generation will turn 65, after which the risk of developing dementia increases exponentially (Bermejo-Pareja et al., 2008). At the same time, the youngest members of this generation are fast approaching 51, the average age of menopause in the United States (Kato et al., 1998). In the past few decades, it has become clear that estrogen signaling plays a significant role in modulating areas of the brain associated with higher cognitive functions, and can have profound neurotrophic and neuroprotective effects in models of injury and disease. The resulting question of how the precipitous drop in circulating estrogen levels during menopause affects the course of cognitive aging in women has become a major focus of research.

Despite this interest, there is still considerable debate as to whether hormone replacement therapy can help to prevent age-related cognitive decline in postmenopausal women. Several laboratory studies in young, cycling women have found that cognitive performance fluctuates with levels of circulating estrogen (E) across the menstrual cycle (Hampson et al., 1990; Phillips and Silverman, 1997; Portin et al., 1999; Postma et al., 1999; Hausmann et al., 2000; Maki et al., 2002; Rosenberg et al., 2002) and across the different phases of birth control (Mordecai et al., 2008), though some studies have failed to replicate these results (Epting and Overman, 1998). Others have found that pharmacological E suppression in premenopausal women can produce measurable cognitive deficits, which are reversed by add-back E treatment (Sherwin and Tulandi, 1996).

Surgical menopause, induced through bilateral oophorectomy, causes a sharp drop in circulating estrogen levels when performed in premenopausal women. Several studies of the effect of bilateral oophorectomy on cognitive function in premenopausal women have found that surgical menopause produces marked deficits across several cognitive domains, which could be prevented by the timely initiation of estrogen replacement therapy in those studies that contained a hormone therapy component (Phillips and Sherwin, 1992; Kimura, 1995; Szklo et al., 1996; Nappi et al., 1999; Verghese et al., 2000; Farrag et al., 2002). However, many clinical studies have failed to reproduce this effect, and it is difficult to determine whether this dichotomy represents a failure of the small sample sizes in the laboratory studies to accurately represent the larger population of surgically menopausal women or a failure of the less-sensitive cognitive assessments performed in the larger studies to detect more subtle declines in cognitive function (Vearncombe and Pachana, 2009).

Evidence suggests that natural menopause does not produce a substantial decline in cognitive function between pre- and post-menopausal women, though a slight dip in learning ability has been reported during perimenopause (Herlitz et al., 2007; Greendale et al., 2009). However, several laboratory studies and randomized clinical trials have found that initiation of hormone replacement therapy (HRT) during perimenopause or soon after the menopausal transition can improve cognitive function (Carlson et al., 2001; Keenan et al., 2001) and reduce a woman’s risk of developing cognitive impairment or dementia later in life (Kimura, 1995; Matthews et al., 1999; Carlson et al., 2001; Zandi et al., 2002; Henderson et al., 2003; Bagger et al., 2005; Henderson et al., 2005; Henderson et al., 2007; Greendale et al., 2009; Rocca et al., 2011).

The literature in this area, however, is far from consistent. Initiation of HRT more than a few years after menopause has been linked to an unchanged or increased risk of dementia and age-associated cognitive decline (Matthews et al, 1999; S.R. Rapp et al., 2003; Shumaker et al., 2003; Henderson et al., 2005; MacLennan et al., 2006). Moreover, several randomized clinical trials have found equivocal or negative effects of HRT on cognitive function, even when initiated soon after menopause (reviewed in Maki and Sundermann, 2009). This inconsistency has left women and their physicians unsure whether and how estrogen replacement therapy should be used in the context of age-related cognitive decline (Buist et al., 2004; Hersh et al., 2004).

Several factors, including hormone formulation, treatment schedule and time between menopause and treatment, have been identified as likely contributors to these discrepancies (reviewed in Sherwin and Henry, 2008). However, determining the optimal parameters for a successful estrogen replacement therapy has been difficult, as the biological mechanisms underpinning the interplay between estrogen, aging and cognitive function are not well understood. The use of an animal model permits the investigation of the effects of estrogen on cognition in tandem with the neurobiological bases of those effects. The nonhuman primate (NHP) is a particularly attractive model in this case, due to the similarity of their reproductive physiology to that of women (Van Esch et al., 2008). These primates have a 28-day menstrual cycle with similar ovarian hormone fluctuations (Dufau et al., 1977) and experience a low-estrogen menopause in their third decade of life (Nichols et al., 2005).

It is worth noting that most research about the effect of estrogen replacement therapy in NHPs is performed on surgically menopausal monkeys. Though there is some evidence that the effect of estrogen therapy may be more robust in women that are surgically menopausal than in naturally menopausal women (Szklo et al., 1996), this is still a matter of considerable debate (reviewed in Vearncombe and Pachana, 2009). Moreover, the same cognitive functions tend to be implicated in both groups as being affected by menopause and by HRT. In NHPs, this same pattern is evident; performance on many of the same tasks is affected by natural and by surgical menopause in aged animals (Roberts et al., 1998; Rapp et al., 2003) and the corresponding changes in the structure of the prefrontal cortex involve many identical features (Hao et al., 2007; Dumitriu et al., 2010). Research in surgically menopausal NHPs, therefore, can provide valuable insight into the mechanisms by which estrogen withdrawal and replacement can affect the function of the brain in order to produce the types of cognitive changes observed after natural menopause.

An additional benefit of the NHP model is the structural and functional similarity of the NHP prefrontal cortex to that of humans (Petrides and Pandya, 1999). The dorsolateral prefrontal cortex (dlPFC) has received increasing interest as a critical site of estrogen's effects on cognitive function. Several studies in young and middle-aged women demonstrate that estrogen improves performance specifically on functions sensitive to the integrity of the dlPFC, such as verbal learning, fluency, and memory, and switching of attention and strategy. Such improvements have been noted during high-estrogen periods of the ovarian cycle (Maki et al., 2002; Rosenberg et al., 2002) and during the estrogen phase of birth control (Mordecai et al., 2008) in premenopausal women. Conversely, estrogen deprivation due to pharmacological blockade (Sherwin and Tulandi, 1996; Berman et al., 1997) or to surgical (Phillips et al., 1992) or natural menopause (Wolf et al., 1999; Keenan et al., 2001) can cause deficits in executive function, which are reversed following add-back estrogen treatment. Such results have led some researchers to propose that the cognitive deficits experienced by postmenopausal women are due to executive dysfunction, and that the PFC, rather than the hippocampus, is the "site of estrogen's effect on cognition" (Keenan et al., 2001).

This review will highlight findings from this NHP model on the interactions among estrogen, the morphological and molecular profiles of dlPFC neurons, and age-related cognitive decline.

2. Cognition

2.1. Measures of cognitive aging in nonhuman primates

Studies have shown that aged NHPs become impaired in both acquiring and performing many dlPFC-dependent cognitive tasks. It is worth noting, however, that the degree of impairment varies considerably among individuals (Presty et al., 1987; Rapp and Amaral, 1991; Herndon et al., 1997), and as such not every cohort is impaired on every task (Rapp et al., 1997; Dumitriu et al., 2010).

Several well-characterized cognitive tasks are commonly used to assess the function of the dorsolateral prefrontal cortex in nonhuman primates. In the delayed nonmatching-to-sample recognition memory task (DNMS), the subject is shown an object, which is removed for a measured delay interval. After this period, the monkey is presented simultaneously with the same object and a new object, and a reward is given for selection of the new object (Rapp et al., 2003). The delayed response test of visuospatial working memory involves two empty wells. The monkey watches as one of the wells is baited, and then a screen descends to hide the wells from the monkey's view for a specified delay interval. After the delay, the screen is raised and the monkey must remember and select the baited well to obtain a reward (Rapp et al., 2003). Given that DR performance critically relies on the integrity of the dlPFC (Gross and Weiskrantz, 1962; Divac and Warren, 1971), and that changes in the morphological and electrophysiological characteristics of area 46 have been demonstrated to correlate with changes in DNMS performance (Peters et al., 1998; Chang et al., 2005; Shamy et al., 2010), these tasks are often used in assessing the effects of aging on the dlPFC in NHPs (Luebke et al., 2010).

In general, aged monkeys have difficulty reversing visual (Rapp, 1990; Voytko, 1999) and spatial (Lai et al., 1995; Herndon et al., 1997) discriminations, performing a monkey version of the WCST, (Moore et al., 2003), and in acquiring (Herndon et al., 1997; Rapp et al., 1997; Dumitriu et al., 2010) and performing (Presty et al., 1987; Rapp and Amaral, 1989; Rapp and Amaral, 1991; Herndon et al., 1997; Rapp et al., 2003; Dumitriu et al., 2010) the DNMS task, especially when the demand on the dlPFC is increased through the use of repeated objects (Rapp and Amaral, 1989). Tests of visuospatial working memory, such as the delayed response task (DR) (Rapp and Amaral, 1989; Roberts et al., 1997; Rapp et al., 2003) and the spatial condition of the delayed recognition span test (spatial-DRST) (Herndon et al., 1997; Moss et al., 1997; Lacreuse et al., 2005), are also highly sensitive to the effects of cognitive aging.

2.2. Effects of estrogen on executive function in nonhuman primates

In intact rhesus monkeys, menopause compounds the effect of aging on cognitive function. Though aging alone affects DR and DNMS task performance, premenopausal monkeys are significantly less impaired than age-matched peri-/postmenopausal animals [see Fig. 1] (Roberts et al., 1997; Hara et al., in press). This pattern is also evident after surgical menopause. Aged intact animals perform substantially better on DNMS than ovariectomized animals of the same age when tested at longer delays (Lacreuse et al., 2000). Estrogen treatment reverses this impairment; aged ovariectomized animals given long-term cyclical estrogen treatment perform the DR task at a level indistinguishable from that of young animals, while those not so supplemented are substantially impaired [see Fig. 2] (Rapp et al., 2003). Estrogen replacement also improves the performance of aged OVX animals on several other age-sensitive tasks, including DNMS (Rapp et al., 2003), the primate analogue

of the WCST (Voytko et al., 2009), and some aspects of a visuospatial attention task (Tinkler and Voytko, 2005; Voytko et al., 2009). In contrast, young animals are resilient to the cognitive effects of estrogen deprivation, and their performance on many of the same visual and spatial memory tasks is unaffected by ovariectomy or estrogen replacement [see Fig. 2] (Voytko et al., 2000; Lacreuse and Herndon, 2003; Hao et al., 2007).

3. Neuronal morphology

It has been demonstrated in recent years that cognitive aging, in the absence of neurodegenerative disorders like Alzheimer's disease, is not associated with significant neuronal loss in the human (Pakkenberg and Gundersen, 1997) or NHP neocortex (Peters et al., 1998). Age-related cognitive decline is thought to result instead from more subtle morphological and molecular changes in the neurons mediating these processes and in the connections between them (Morrison and Hof, 1997; Hof and Morrison, 2004).

3.1. Spine/synapse density

3.1.1. Effect of aging on prefrontal spine/synapse density—The great majority of excitatory synapses between cortical neurons occur on tiny dendritic protrusions called spines (Nimchinsky et al., 2002). These excitatory synapses are referred to as "asymmetric" based upon their appearance when viewed under an electron microscope. Pyramidal neurons in the human PFC lose a significant proportion of their dendritic spines with age (Jacobs et al., 1997), both in terms of the number of spines per neuron and the density of spines per unit dendritic length. The same pattern is evident in NHPs. Between young adulthood and old age there is a significant decrease in the density of both dendritic spines and asymmetric synapses in the dlPFC of rhesus monkeys. This loss is evident in the NHP dlPFC in layers II/III and V (Uemura, 1980; Duan et al., 2003; Peters et al., 2008), across the apical and basal dendritic trees of the pyramidal neurons in layers III and IV (Uemura, 1980; Duan et al., 2003; Hao et al., 2007; Dumitriu et al., 2010), and is especially profound in layer I, where 50% of axospinous synapses may be lost (Peters et al., 1998).

This dramatic decrease in prefrontal connectivity has a measurable impact on cognitive performance. In fact, the degree of spine or synapse loss in the dlPFC has been found to correlate with individual age-related cognitive impairment on several tasks, including DNMS acquisition (Peters et al., 1998; Dumitriu et al., 2010) and recognition accuracy (Peters et al., 1998) and performance on the spatial condition of the DRST (Peters et al., 1998).

3.1.2. Effect of estrogen on prefrontal spine/synapse density—In contrast to the effect of aging, cyclical estrogen treatment in the form of a single injection of 17β-estradiol administered every three weeks substantially increases dendritic spine density in the dlPFC in both young and aged OVX monkeys [Fig. 2A] (Tang et al., 2004; Hao et al., 2006; Hao et al., 2007). Estrogen also increases asymmetric synapse density in this area, suggesting that many of these new spines form synaptic contacts (Leranth et al., 2008). In aged animals, this increase is accompanied by improvements in DR and DNMS performance compared to untreated controls (Rapp et al., 2003). Interestingly, dendritic length increases in young OVX animals without estrogen treatment, such that the number of spines per neuron remains approximately stable despite the drop in spine density (Hao et al., 2007). No such outgrowth occurs in estrogen-deprived aged animals. It is possible that this represents one mechanism by which the young brain is capable of adapting to the lack of estrogen and preserving cognitive function.

3.2. Effects of aging and estrogen on dendritic spine morphology

Dendritic spines are highly variable in shape and size (Harris et al., 2002). Studies using *in vivo* time-lapse imaging have shown that it is possible to separate these spines into independent populations based on their morphology and average lifespan (Holtmaat et al., 2005). Large mushroom spines are remarkably persistent *in vivo*, lasting for months, years, or potentially for the life of the animal (Kasai et al., 2003; Holtmaat et al., 2005). These spines are characterized by large postsynaptic densities (Harris et al., 1992), with large numbers of AMPA receptors (Matsuzaki et al., 2001). In contrast, long, thin spines have a high rate of turnover, and are continually formed both at rest and during activity-dependent processes (Kasai et al., 2003). These spines have few AMPA receptors (Matsuzaki et al., 2001) but abundant NMDA receptors (Noguchi et al., 2005) and are highly motile, capable of retracting back into the dendrite or stabilizing and expanding to form new mushroom spines (Kasai et al., 2003; Holtmaat et al., 2005). Long-term potentiation (LTP) and long-term depression (LTD) provide a mechanism for conversion between these two classes of spines. LTP induction causes a rapid and persistent enlargement of thin spines and an increase in functional AMPA receptor expression (Matsuzaki et al., 2004), while LTD leads to spine shrinkage and retraction (Zhou et al., 2004).

It has been proposed that mushroom and thin spines serve as “write-protected” and “write-enabled” bits of memory, respectively (Kasai et al., 2003). The stability and strength of the mushroom spine makes it a good candidate for the storage of long-term memories, while the labile, dynamic thin spine population provides an ever-refreshing candidate pool for the formation of new connections.

3.2.1. Effects of aging on spine morphology—Not all spine types are lost equally with age; in fact, the density of mushroom spines, defined as those with a head diameter greater than 0.6 μm, does not decrease with age in NHP dlPFC (Hao et al., 2007; Dumitriu et al., 2010). Instead, the age-related decrease in spine density is driven almost entirely by the loss of thin spines, in both intact and ovariectomized animals [Fig 3B, Fig 4A] (Hao et al., 2007; Dumitriu et al., 2010). It is the density of these thin spines, in particular, that drives the correlation between spine density and DNMS acquisition (Dumitriu et al., 2010).

It has also been noted that those thin spines which remain in aged animals are larger than those present in younger animals [Fig. 4B] (Dumitriu et al., 2010). Thin spines grow larger slowly after their formation, and so the size of these spines may reflect the rate of their turnover (Yasumatsu et al., 2008), with larger thin spines indicating a slower rate. The size of these spines is the strongest predictor of individual cognitive performance in aged surgically-intact animals [Fig. 4C] (Dumitriu et al., 2010), suggesting that maintaining a high rate of turnover in the thin spine population is particularly critical for optimal function of the dlPFC.

3.2.2. Effects of E on spine morphology—Estrogen treatment selectively increases the density of these vulnerable thin spines both *in vitro* and *in vivo*. Addition of estrogen to rat CA1 hippocampal neurons increases the number of thin spines and filipodia without changing the numbers of mushroom or stubby spines (Mukai et al., 2007). In NHPs, the increase in spine density on layer III pyramidal neurons in the dlPFC following cyclical E replacement occurs primarily in the smallest 25% of spines, restoring the pool of thin “learning” spines that are vulnerable to aging and connected to age-related cognitive impairment [Fig. 3B] (Hao et al., 2006, 2007). The cyclical increase and loss of spines with naturally fluctuating estrogen levels (Woolley et al., 1990) may serve to maintain a high turnover rate in this spine population, refreshing the pool of potential synaptic contacts.

These results highlight the increased vulnerability of aged animals to the loss of circulating estrogen. As aging and estrogen deprivation both reduce the population of thin spines in the dlPFC, the combination of these states produces a “double hit” effect that results in a 3-fold difference in the density of thin spines between young estrogen-treated animals and aged animals lacking E [Fig. 3B] (Hao et al., 2007).

4. Effects of aging and estrogen on the synaptic molecular profile

In addition to understanding the effects of estrogen on connectivity in the PFC, it is critical to understand the mechanisms by which these effects are produced if the cognitive benefits of estrogen therapy seen in primate studies are to be successfully brought to clinical practice. Using postembedding immunogold electron microscopy, it is possible to view the distribution of a protein in individual synapses through the use of antibodies linked to electron-dense gold particles (see Wang et al., 2010 for detailed methods). We have begun to link certain molecular attributes of excitatory synapses in the dlPFC to E effects and cognitive performance in young and aged rhesus monkeys.

4.1. The role of synaptic estrogen receptor α in estrogen signaling and cognition

The classical estrogen receptors, ER-α and ER-β, are DNA-binding transcription factors which are generally located in the nucleus and cytosol (Htun et al., 1999). In recent years it has become clear that membrane-bound forms of both ER-α (Milner et al., 2001; Adams et al., 2002) and ER-β (Milner et al., 2005) are present in dendritic spines and presynaptic terminals, strategically positioned to modulate both transmitter release and spine dynamics. These receptors can rapidly modulate synaptic structure and function through the activation of second-messenger systems (Toran-Allerand, 2000; Dominguez et al., 2007), and can be activated both by circulating gonadal estrogen and estrogen synthesized locally within neurons (Hojo et al., 2008).

Activation of synaptic ER-α appears to be necessary for the effect of estrogen on the volumetric density of spine and synapse in the rat hippocampus. Selective ER-α activation is significantly more effective than that of ER-β at mimicking estrogen’s spinogenic effect in CA1 neurons, and the addition of an ER-α antagonist blocks this spinogenic response (Mukai et al., 2007). In female rats, the percentage of hippocampal synapses containing ER-α decreases sharply with age [Fig. 5] (Adams et al., 2002), as does the effect of estrogen on hippocampal spine density (Adams et al., 2001). Estrogen treatment has no effect on hippocampal ER-α levels in either young or aged OVX rats [Fig. 5] (Adams et al., 2002).

ER-α is also located both pre- and postsynaptically in the NHP dlPFC (Wang et al., 2010). In contrast to the effect in the rat hippocampus, synaptic ER-α levels in the NHP remain stable with age, though these levels are similarly unaffected by estrogen status [Fig. 5] (Wang et al., 2010). Despite the stability in overall ER-α prevalence across age and estrogen treatment groups, examination of the synaptic localization and abundance of this receptor in the context of individual cognitive performance reveals striking correlations.

4.2. ER-α and cognition in young OVX animals in the absence of estrogen

The distribution of synaptic ER-α in the dlPFC of young rhesus monkeys predicts individual cognitive resilience to estrogen withdrawal after surgical menopause. Higher levels of ER-α in the presynaptic terminal are strongly correlated with higher average accuracy on the DR task in young animals not given estrogen treatment after OVX (Wang et al., 2010). This additional presynaptic ER-α may allow young animals to make the best possible use of the more limited amounts of estrogen available in the absence of circulating gonadal estrogen. In addition, the presence of ER-α in perforated synapses appears to be of particular significance. Perforated synapses are those marked by the presence of a discontinuity within

the postsynaptic density. Synapses with perforations tend to be particularly large, stable, and strong, with a significantly higher density of synaptic AMPA receptors than non-perforated synapses of the same size (Ganeshina et al., 2004). The percentage of perforated synapses containing ER-α correlates strongly with DR performance in estrogen-deprived young animals (Wang et al., 2010), with higher percentages predicting better accuracy. Perforated synapses tend to be very large and stable, with a higher density of AMPA receptors than non-perforated synapses (Ganeshina et al., 2004). Thus, perforated synapses are proposed to be a structural correlate of enhanced synaptic efficacy (Peters and Kaiserman-Abramof, 1969; Greenough et al., 1978; Geinisman et al., 1991). This may indicate that, when circulating estrogen is unavailable, young animals are able to compensate for the unavailability of thin spines by strengthening existing large, stable synapses (Wang et al., 2010). The absence of such correlations in the aged estrogen-deprived animals (Wang et al., 2010) may reflect the inability of these animals to compensate for the loss of E, as evidenced by their poor DR performance (Hao et al., 2007).

4.3. ER-α and cognition in aged OVX animals after estrogen treatment

Synaptic ER-α distribution also predicts the magnitude of cognitive improvement experienced by individual aged animals given estrogen replacement therapy, as compared to aged animals without E. Increased abundance of postsynaptic ER-α located 30–60 nm from the synaptic membrane is highly correlated with improved DR performance in those aged animals given estrogen treatment after OVX (Wang et al., 2010). Receptors located in this region are well-positioned to activate the second messenger cascades involved in ER-mediated spine and synapse formation (Spencer et al., 2008).

4.4. Implications for hormone replacement therapy in women

There is considerable variability in the cognitive response of individual women to both estrogen depletion and estrogen replacement therapies (Ancelin and Ritchie, 2005). These findings open up a new avenue by which to understand the mechanisms behind that variability, which seems especially promising in light of recent studies linking polymorphisms of the ER-α gene ESR1 to differential risk of developing dementia (Sundermann et al., 2010).

In addition to ER-α, the estrogen receptors ER-β and GPR30 have been implicated in estrogen's effects on cognitive function in rodent models. As mentioned above, ER-β is also localized in rat hippocampal spines and presynaptic terminals (Milner et al., 2005). Administration of specific ER-β agonists to OVX rats replicates the effects of estradiol on performance of several cognitive tasks (Jacome et al., 2010; Neese et al., 2010), and ER-β signaling may be especially important in mediating the anxiolytic effects of estrogen (reviewed in ter Horst, 2010). GPR30, a recently characterized estrogen receptor, is present in the rat hippocampus and frontal cortex (Hazell et al., 2009; Hammond et al., 2011), and localizes to the plasma membrane in rat hippocampal pyramidal neurons (Funakoshi et al., 2006). GPR30 signaling has been implicated in estrogen's neuroprotective effects in hippocampal cells (Gingerich et al., 2010). Specific agonists of ER-α, ER-β and GPR30 have all been reported to replicate the beneficial effect of estradiol on acquisition of a spatial learning task when administered to OVX rats (Hammond et al., 2009).

These results suggest that targeting ER-α, and perhaps other synaptic estrogen receptors, may provide a promising alternative to global estrogen replacement as a means to preserve cognitive function in naturally and surgically menopausal women.

5. Conclusion

The work described above has led to a useful framework and set of hypotheses regarding NHP dlPFC that links synaptic plasticity, cognitive aging, and interactive effects of estrogen. We hypothesize that the cognitive domains mediated by dlPFC require a particularly high degree of synaptic plasticity for axospinous synapses. We hypothesize further that a key element of such plasticity is the outgrowth of new, thin, highly motile, potentially transient spines that are available for synaptic stabilization in the context of learning. Cognitive decline is linked to age-related loss of such spines and E-enhanced cognition in aged monkeys occurs through partial restoration of this spine/synapse class. Importantly, the effect of E on this class of synapses suggests that a substantial proportion of age-related spine loss is not inevitable and may be responsive to pharmaceutical intervention. Such approaches may be particularly important if age-related synaptic alterations such as those described here leave these neurons vulnerable to the degenerative cascade that occurs in AD in humans. In addition, the findings regarding synaptic ER-α suggest that strategies targeting the synaptic ERs directly rather than relying exclusively on E replacement may be useful for cognitive enhancement in women.

The NHP model has provided several novel insights into our understanding of the effects of estrogen on the structure and function of the dlPFC in the context of aging. This research has already led to promising new directions for research into safer, better-targeted therapies for menopausal women, as well as to a new understanding of the mechanisms behind individual variation in cognitive ability. Our current and future research with the NHP model will proceed along several related paths. For example, great effort will be directed toward defining the molecular profile of thin vs. mushroom spines to reveal potential targets to enhance plasticity and protect against age-related cognitive decline. In addition, other treatment regimens (e.g., chronic, combined with progesterone, etc.) are being tested to determine if they are as effective at rescuing the target spine class and sustaining cognitive function as is cyclical, unopposed E, which was the treatment used in the studies described above. In addition, we will investigate the importance of the timing of E treatment with respect to both a potential window of opportunity after the cessation of ovarian function and the duration of cognitive enhancement once treatment is discontinued. These NHP studies will have a particularly high degree of translational power and will directly inform treatment issues in women.

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Abbreviations

NHP	nonhuman primate
E	estrogen/estradiol
DR	delayed response
DNMS	delayed nonmatching to sample
DRST	delayed recognition span test
OVX	ovariectomized

dlPFC	dorsolateral prefrontal cortex
ER-α	estrogen receptor alpha
WCST	Wisconsin card sorting task
PFC	prefrontal cortex
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA	n-methyl-d-aspartic acid
LTP	long-term potentiation
LTD	long-term depression
CA1	Cornu Ammonis area 1
ER-β	estrogen receptor beta

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Research Highlights

- Young monkeys lacking estradiol perform as well as estradiol-treated monkeys.
- Aged OVX animals given estradiol perform as well as young adults.
- Aging decreases thin spines on pyramidal neurons in the prefrontal cortex.
- Estradiol increases the proportion of thin spines in the prefrontal cortex.
- Thin spines correlate with cognitive performance.

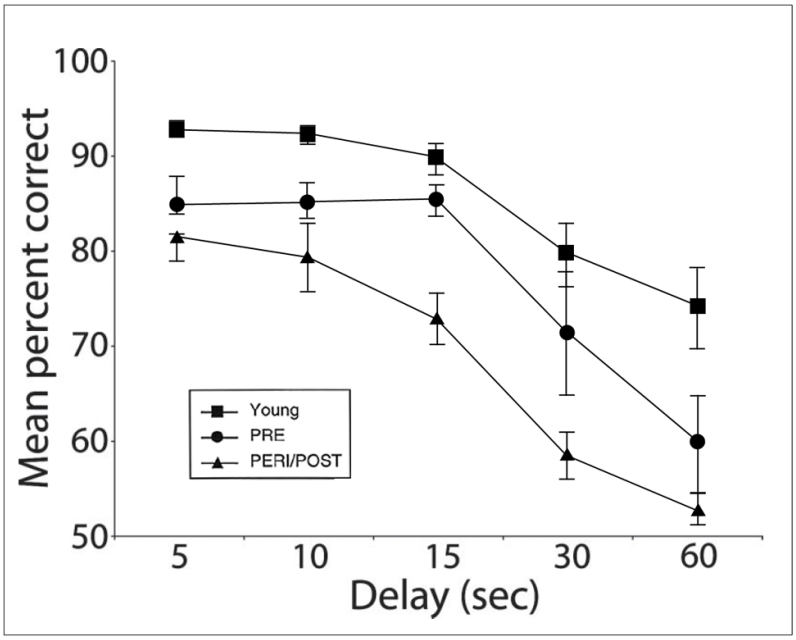

Figure 1. DR performance in young and aged surgically-intact Rhesus monkeys

Young monkeys perform the DR task with higher average accuracy than aged animals ($p < 0.05$). Among aged animals, pre-menopausal monkeys perform more accurately than age-matched peri-/postmenopausal monkeys ($p < 0.05$). (PRE = pre-menopausal, PERI/POST = peri-/postmenopausal; reproduced from Roberts et al., 1997)

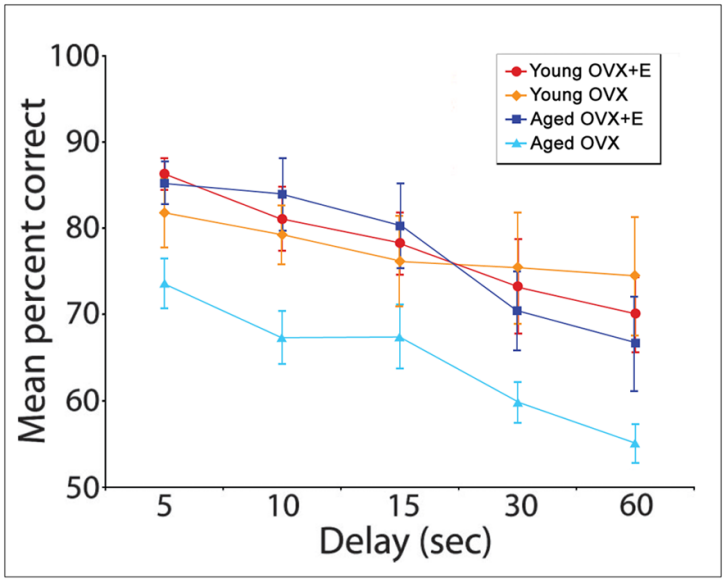

Figure 2. DR performance in young and aged OVX Rhesus monkeys with and without E replacement

Young OVX animals perform the DR task with equivalent average accuracy whether treated with cyclical estrogen therapy or with vehicle alone. Aged OVX monkeys perform significantly less accurately than young animals ($p < 0.03$ at all time points), but this deficit is reversed by cyclical estrogen treatment. (E = estrogen-treated, other animals are treated with vehicle alone; reproduced from Hao et al., 2007)

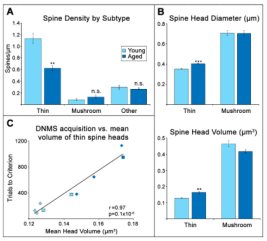

Figure 3. Spine density and head diameter in young and aged OVX Rhesus monkeys with and without E replacement

Effect of estrogen treatment on dendritic spines on dlPFC pyramidal neurons in young and aged OVX monkeys. A. Estrogen treatment increases spine density on dlPFC neurons in OVX monkeys. B. Estrogen treatment also increases the proportion of smaller spines in young and aged OVX monkeys. (E = estrogen-treated, Veh = vehicle-treated; adapted from Hao et al., 2007)

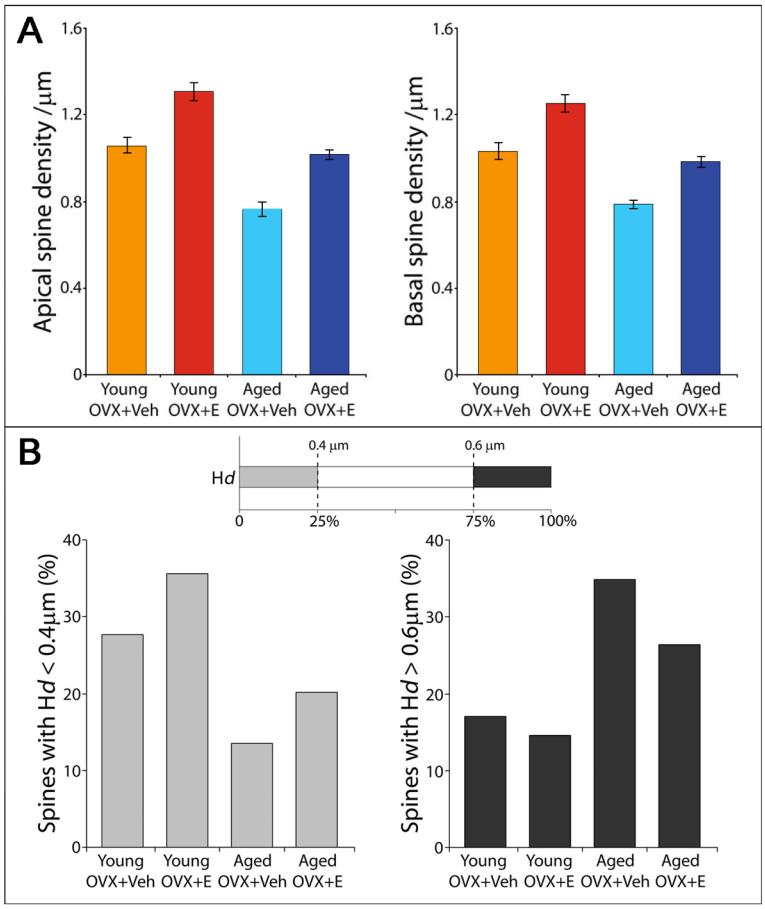

Figure 4. Density and size of thin and mushroom spines in young and aged surgically-intact Rhesus monkeys and their correlation with the speed of DNMS acquisition

A. The density of thin spines, but not of mushroom spines, is decreased in the dlPFC of aged monkeys. B. Thin spines in the aged NHP dlPFC are larger on average than those in young monkeys. C. The mean volume of thin spines is highly correlated with the number of trials required to learn the DNMS task in both aged and young monkeys. (adapted from Dumitriu et al., 2010)

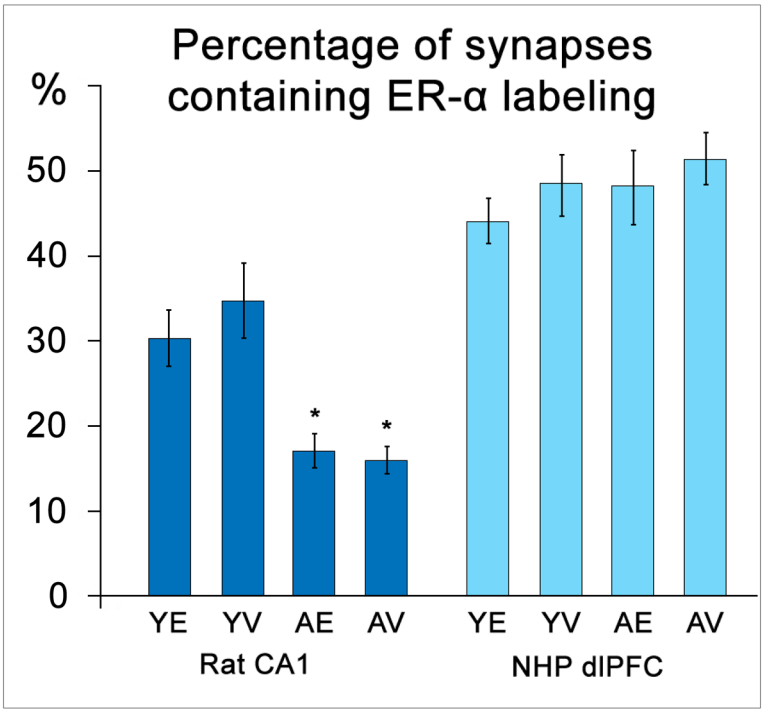

Figure 5.

Percentage of synapses labeled for ER-α in the rat CA1 and NHP dlPFC in the context of age and estrogen status. Unlike levels in the rat hippocampus ($p < 0.0001$ for both aged groups), ER-α levels in the monkey dlPFC do not decline with age ($p > 0.05$). (Y = young, A = aged, E = estrogen-treated, V = vehicle-treated; adapted from Adams et al., 2002 and Wang et al., 2010)