Cumulative Estrogen Exposure and Prospective Memory in Older Women

By

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ABSTRACT

With the average female life expectancy in Canada currently at 82 years, women are now spending approximately one-third of their lives in a hypoestrogenic state. Numerous studies from the basic sciences have shown that estrogen is neuroprotective in a variety of ways. The findings from the clinical studies of the effects of estrogen replacement on cognition in postmenopausal women are more inconsistent, though current research suggests that the timing of initiation of estrogen replacement relative to menopause is a major contributing factor to the discrepant findings in this literature. Reproductive and biological variables that affect levels of endogenous estrogen throughout a woman's lifespan may also influence cognitive function later in life as well as modify a woman's response to exogenous estrogen.

The present study looked at the effects of cumulative estrogen exposure on the performance of a measure of prospective memory in a group of older postmenopausal women. Cumulative estrogen exposure was estimated using a mathematical index that included variables known to influence estrogen levels across the life span such as age at menarche and menopause, parity, duration of breastfeeding and estrogen replacement therapy, body mass index and time

since menopause. Prospective memory is memory for future intentions and it was chosen because of its relevance for independent function and possible utility as an early indicator of dementia. Imaging studies link prospective memory to the prefrontal cortex, an area of the brain that is known to be influenced by estrogen so it was hypothesized that women with higher levels of cumulative estrogen exposure would perform better on a measure of prospective memory than women with lower levels of endogenous estrogen. Measures of verbal memory were also included in the study for comparative purposes as verbal memory is the cognitive function reported most consistently in the previous literature to be affected by postmenopausal estrogen replacement use.

The results of the multivariate analyses indicated a significant positive correlation between prospective memory scores and cumulative estrogen exposure but only when women who had initiated estrogen replacement more than five years after menopause were excluded from the analysis. The significant positive correlation between cumulative estrogen exposure and prospective memory performance remained significant when only women who had never used estrogen were included in the analysis. No relationships were observed between levels of cumulative estrogen exposure and performance on measures of verbal memory. While the study was limited by the fact that it was observational in nature and the sample size was small, the results are consistent with recent findings from the hormone literature, in that they suggest that the timing of hormone replacement as well as the influence of variables that affect endogenous

estrogen levels over a woman's lifespan need to be considered when studying relationships between cognitive performance and estrogen. In addition, the finding that performance on a measure of prospective memory but not performance on a measure of verbal memory was associated with levels of cumulative estrogen exposure adds further support to the theory that the frontal cortex may be especially sensitive to estrogen.

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Acknowledgements

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Dedication

This dissertation is dedicated to the memory of mother, Dr. Elizabeth Hesson.

Chapter 1

INTRODUCTION

Estrogen is a steroid hormone primarily produced by the ovaries (Osterlund & Hurd, 2001). Although it has been well established that estrogen functions as a reproductive hormone (McEwen & Alves, 1999), a substantial amount of research has emerged in the last 25 years to suggest that estrogen's effects in the adult brain extend beyond influencing reproductive behavior. The current literature indicates that estrogen is important in maintaining neuronal functioning and modulating brain physiology (Palacious, Cifuentes, Menedez, & Von Helde, 2000) and may have a role to play in psychiatric conditions such as Schizophrenia (Maric, Popovic, Jasovic-Gasic, Pilipovic & van Os, 2005; Rao & Kolsch, 2003) and Major Depressive Disorder (Perlman et al, 2005a) as well as neurodegenerative conditions such as Alzheimer's Disease (Brinton, 2004) and Parkinson's Disease (Currie, Harrison, Trugman, Bennett & Wooten, 2004). Of particular relevance for the current investigation are the numerous basic science and clinical studies that report a significant impact of estrogen on behavioral measures of cognitive function as well as the neuroanatomical structures believed to underlie these functions. The aim of this first chapter is to provide a review of this literature.

Estrogen Actions in the Brain

Although the cellular and molecular mechanisms underlying estrogen's actions in the brain have yet to be fully elucidated, it is thought that many, but not all, of estrogen's effects are mediated through the estrogen receptor (ER; Deroo & Korach, 2006; Zhao, Wu, & Brinton, 2004). To date, two ER receptors have been identified, ERα and ERβ (Osterlund & Hurd, 2001). Both of these receptors have been detected in the human hippocampus and cerebral cortex (Osterlund, Gustafsson, Keller, & Hurd, 2000; Osterlund & Hurd, 2001; Perlman et al, 2005a; Perlman et al, 2005b; Savaskan, Olivieri, Meier, Ravid, Muller-Spahn, 2001), making a role for these receptors in memory and other cognitive functions quite plausible (Osterlund et al., 2000). In fact, the results of recent studies suggest that ERα gene polymorphisms may predispose a person to cognitive impairment (Olsen et al, 2006; Yaffe, Lui, Rad, & Morin, 2002).

Neurotrophic Actions of Estrogen

Estrogen has a number of effects on the morphology and electrophysiology of neurons in the hippocampus, a structure that is considered essential for declarative memory (Squire, 1992). In rodents, ERα and ERβ have been found in the dendritic spines and axon terminals of hippocampal neurons (Milner et al, 2005; Milner et al, 2001).

In the mammalian brain, dendritic spines represent a means by which new connections can be established between neurons and existing connections can be strengthened (Leuner, Faltudo, & Shors, 2003). There is some evidence to

suggest that changes in dendritic spine morphology may be involved in learning and memory (Nimchinsky, Sabatini & Svoboda, 2002).

In rodents, ovariectomy significantly reduces dendritic spine density in the hippocampus (Gould, Woolley, Frankfurt, & McEwen, 1990). Administration of estrogen subsequent to ovariectomy prevents this decrease (Gould et al, 1990) and also increases hippocampal synapse density in treated rats relative to rats that do not receive estrogen (Woolley & McEwen, 1992). In addition, the density of synapses in the hippocampus varies with natural fluctuations in estrogen levels during the 5-day estrus cycle of the rodent such that a greater density of synapses is found on days when estrogen levels are higher (Woolley & McEwen, 1992). Kinsley et al (2006) recently reported that rodent brains show a proliferation of dendritic spines in the hippocampus during pregnancy, a period when estrogen levels remain elevated. Increases in the density of dendritic spines and spine synapses have also been reported in ovariectomized nonhuman primates treated with estrogen relative to untreated animals (Hao et al, 2003; Leranth, Shanabrough, & Redmond, 2002). From a functional point of view, estrogen induced increases in hippocampal spine density have been shown to correspond with improvements in spatial learning in rodents (Garza-Meilandt, Cantu, & Claiborne, 2006).

In terms of electrophysiology, exogenous and endogenous estrogen have both been found, in rodents, to facilitate hippocampal long-term potentiation (LTP), a form of synaptic plasticity thought to be involved in learning and

memory (Cordoba Montoya & Carrer, 1997; Good, Day, & Muir, 1999; Warren, Humphreys, Juraska, & Greenough, 1995). Estrogen-induced increases in LPT appear to be due to estrogen's ability to rapidly increase calcium-calmodulin-dependent protein kinase II (CaMKII) activity in the hippocampus (Sawai et al, 2002).

Estrogen has also been shown to have effects on the structure of neurons in the prefrontal cortex (PFC). The PFC subserves cognitive processes such as working memory, selective attention and behavioral inhibition (see Miller & Cohen, 2001 for a review). Dendritic spine number and density in the PFC have been found to decrease with age in humans (Jacobs, Driscoll, & Schall, 1997), a finding that is consistent with reports of frontal atrophy in the aging human brain (Alexander et al, 2006; Brickman et al, 2006). In comparison to intact animals, female rats that undergo ovariectomy exhibit a reduction in dendritic spine density in the PFC that is accompanied by deficits in the performance of memory tasks (Wallace, Luine, Arellanos & Frankfurt, 2006). In ovariectomized nonhuman primates, the administration of estrogen increases dendritic spine density in the PFC (Hao et al, 2006; Tang et al, 2004). This estrogen induced increase in spine density has been reported to occur in conjunction with improvements in a delayed response test of spatial working memory in the same animals (Rapp, Morrison, & Roberts, 2003).

In summary, estrogen is capable of modifying neuronal properties in the hippocampus and PFC of rodents and nonhuman primates. Given the relevancy

of the hippocampus and PFC for human cognition, these findings support a role for estrogen in cognition.

Estrogen and Neurotransmitters

Estrogen also affects a number of the neurotransmitter systems in the brain with the cholinergic, serotonergic and dopaminergic systems all being responsive to estrogen (McEwen & Alves, 1999). The human basal forebrain contains cholinergic neurons that provide the major cholinergic innervation to the neocortex and hippocampus where they are involved in cognitive function (Mufson, Ginsberg, Ikonomovic, & Dekosky, 2003). Consistent with this are the clinical findings that changes in cholinergic function show the strongest correlation with the cognitive decline associated with aging (Araujo, Studzinski, & Milgram, 2005) and that loss of forebrain cholinergic neurons contributes to the dementia seen with Alzheimer's Disease (AD; Gibbs, 1999; Oddo & Laferla, 2006).

In rodents, lesions of the basal forebrain cholinergic system produce extensive cholinergic loss and a corresponding impairment on tasks of learning and memory (Conner, Culberson, Packowski, Chiba, & Tuszynski, 2003; Paban, Chambon, Jaffard, & Alescio-Lautier, 2005; Vale-Martinez, Baxter, & Eichenbaum, 2002). Ovariectomy has been found to reduce high-affinity choline uptake (HACU) and choline acetyltransferase activity (ChAT), the rate-limiting enzyme for acetylcholine formation, in the frontal cortex and hippocampus of rodents (Singh, Meyer, Millard, & Simpkins, 1994). Animals given estrogen

following ovariectomy show enhanced cholinergic function relative to untreated animals as evidenced by increases in HACU (Gibbs, 2000; Singh et al, 1994), enhancement of potassium-stimulated acetylcholine release (Gabor, Nagle, Johnson, & Gibbs, 2003; Gibbs, Hashash, & Johnson, 1997), less reduction in ChAT activity following ovariectomy (Singh et al, 1994), and enhancement of hippocampal acetylcholine release during maze learning (Marriott & Korol, 2003). There is also evidence to suggest that estrogen can reduce cognitive deficits associated with impaired cholinergic function. Rodents who receive estrogen following ovariectomy also show a reduction in performance deficits on spatial and working memory tasks, relative to animals who do not receive estrogen, following administration of scopolamine, an anticholinergic drug (Fader, Hendricson, & Dohanich, 1998; Fader, Johnson, & Dohanich, 1999; Gibbs, 1999; Tanabe, Miyasaka, Kubota, & Aso, 2004).

In nonhuman primates, ovariectomy results in a decrease in cholinergic fiber density in the PFC that can be prevented by the administration of estrogen (Kritzer & Kohama, 1999; Tinkler, Tobin, & Voytko, 2004). Both lesions of the basal forebrain cholinergic system and administration of scopolamine impair aspects of visual attention in monkeys (Voytko et al, 1994). However, scopolamine's effects on visual attention appear to be sensitive to the presence or absence of estrogen (Voytko, 2002).

Neuroendocrine challenge is a technique that provides an indirect method through which to study the effects of estrogen on neurotransmitter tone in

humans (Craig et al, 2004). Growth hormone response to pyridostigmine, an acetylcholinesterase inhibitor, is one method of measuring cholinergic function in humans (van Amelsvoort et al, 2003). Van Amelsvoort et al (2003) report that, in comparison to estrogen naïve participants, women in their study who had taken estrogen for a number of years had a significantly larger release of growth hormone following pyridostigmine, suggesting that estrogen increased central cholinergic tone in these women. Dumas, Hancur-Bucci, Naylor, Sites, and Newhouse (2006) recently looked at the interaction between estrogen and the cholinergic system and the effects of this interaction on cognition in healthy postmenopausal women. Women randomly received 3 months of either estrogen or placebo following which they completed five days of "cholinergic challenge" where they were given scopolamine, mecamylamine or placebo. Mecamylamine, like scopolamine, is an anticholinergic drug. After these five days, women were then crossed over to the other condition of either placebo or estrogen for another 3 months and then completed five more challenge days. Neuropsychological testing was completed on each of the challenge days and consisted of a battery of tests that looked at attention, psychomotor speed as well as verbal and nonverbal learning and memory. Equivalent versions of the testing forms were created so that a new version of each test was used for each test day. The results of the study showed that administration of scopolamine or mecamylamine impaired performance on a number of the cognitive tasks and that administration of

estrogen attenuated this impairment on measures of attention and psychomotor speed.

Smith, Minoshima, Kuhl, and Zubieta (2001) used single photon emission computed tomography (SPECT) to look at the effects of long-term hormone use on concentrations of cholinergic synapses in the brains of healthy postmenopausal women. A positive correlation was found between length of estrogen replacement therapy and cholinergic synaptic concentration in the frontal, parietal and temporal cortices. In women who did not take estrogen, age at menopause was positively correlated with cholinergic synaptic concentration in the temporal cortex. The results suggest that duration of exposure to endogenous and exogenous estrogen may be relevant for the survival of cholinergic neurons in postmenopausal women (Smith et al, 2001).

In a recent study, Norbury et al (2007) reported that, in comparison to never users, women who were long-term users of estrogen had significantly higher muscarinic acetylcholine receptor density in the left striatum and hippocampus, lateral frontal cortex and thalamus. A significant correlation was also found between muscarinic acetylcholine receptor density in the hippocampus and temporal and circulating estrogen levels in the group of women who were long-term users of estrogen. While no relationship was found between muscarinic acetylcholine receptor density and cognitive function, long-term users of estrogen were noted to have made significantly fewer perseverative errors on the California Verbal Learning Test (CVLT) than nonusers. Norbury et

al (2007) suggest that estrogen's proposed neuroprotective effect against cognitive aging and AD may be in part due to its ability to affect the aging of muscarinic acetylcholine receptors in the brain.

Estrogen's ability to influence acetylcholinergic and cognitive function may be due, in part, to its effects on the neurotophin, brain-derived neurotophic factor (BDNF). BDNF is a member of the neurotrophin family of proteins that promote the growth, survival and maintenance of neurons (Lee & McEwen, 2001). BDNF has been shown to increase levels of acetylcholine in rodent basal forebrain cultures (Auld, Mennicken, Day, Quirion, 2001) and there is also considerable evidence from basic science to support a role for BDNF in hippocampal LTP (see Blum & Konnerth, 2005 for a review). Clinical studies have found that individuals with the val66met polymorphism of the BDNF gene, which results in reduced secretion of BDNF (Chen et al, 2004; Egan et al, 2004), have poorer episodic memory (Egan et al, 2004; Ho et al, 2006), significant reductions in gray matter volumes in the hippocampus and PFC (Ho et al, 2006; Pezawas et al, 2004) and greater susceptibility to psychiatric conditions such as schizophrenia (Rosa et al, 2006) and bipolar disorder (Neves-Pereira et al, 2002) than individuals without this polymorphism. BDNF is also reduced in individuals with AD and appears to be associated with the cognitive decline that is seen in this disorder (Holsinger, Schnarr, Henry, Castelo & Fahnestock, 2000; Peng, Wuu, Mufson & Fahnestock, 2005; Siegal & Chauhan, 2000). In rodents, ovariectomy produces a significant decrease in BDNF in the frontal and temporal

cortices as well as the hippocampus (Singh, Meyer, & Simpkins, 1995). In comparison to untreated animals, ovariectomized rats that receive estrogen show significant increases in levels of BDNF in the hippocampus (Zhou, Zhang, Cohen & Pandey, 2005) as well as enhanced retrograde transport of BDNF in the forebrain (Jezierski & Sohrabji, 2003).

Serotonin is another neurotransmitter that appears to be relevant for cognition. On postmortem, the brains of AD patients with severe dementia show an increased loss of serotonin neurons in the frontal and temporal cortices relative to less demented patients whose receptor densities resemble those of controls (Lai et al, 2005). In addition, the degree of serotonin receptor loss in the temporal cortex of AD patients has been found to correlate with the rate of cognitive decline observed in the patients (Lai et al, 2005).

Acute tryptophan depletion (ATD) has been used to study central serotonergic function and cognition in healthy humans. ATD lowers serotonin levels by depleting its amino acid precursor, tryptophan (Riedel, Klaassen, & Schmit, 2002). Deficits in learning and memory consolidation (Harrison et al, 2004; Riedel et al, 2002; Schmitt et al, 2000) and improvements in focused attention and executive function have been reported in healthy subjects following ATD (Evers, Van Der Veen, Jolles, Deutz, & Schmitt, 2006; Riedel et al, 2002; Scmitt et al, 2000). Loading of tryptophan, which increases serotonin levels, has been shown to negatively impact working memory as evidenced by a reduced number of digits recalled in reverse order following loading versus

baseline in healthy subjects (Luciana, Burgund, Berman, & Hanson, 2001). Improvements seen in certain cognitive functions with a reduction in serotonin may reflect the removal of serotonin inhibitory influences in the cortex (Robbins, 1997; Schmitt et al, 2000).

Estrogen appears to interact with serotonin to affect cognition. Amin et al (2006) recently looked at the effects of estrogen replacement on cognition in menopausal women undergoing ATD. Women completed ATD and cognitive testing before and after 8 weeks of estrogen replacement. The results of the study showed that ATD impaired performance on measures of verbal memory before administration of estrogen but not after 8 weeks estrogen treatment. Neither ATD nor estrogen had an effect on measures of visuospatial memory. Amin et al (2006) state that their results indicate that estrogen is able to enhance serotonin activity in regions of the brain that are relevant to cognition.

The results of a study by Kugaya et al (2003) support Amin et al's (2006) conclusions. Kugaya et al (2003) used positron emission tomography (PET) to study the effects of estrogen replacement on serotonin receptors in the brains of postmenopausal women. Women in their study underwent PET measurements of serotonin receptor binding as well as cognitive assessment before and after being given estrogen. The results of the study showed an increase in serotonin receptor binding in the right frontal cortex following the administration of estrogen. In addition, significant improvements in performance on

executive/prefrontal-related functions (e.g., verbal fluency and Trail Making Test A) were observed following estrogen replacement.

A number of recent imaging studies indicate a role for striatal dopaminergic activity in executive functions such as working memory, planning, and attention (Mehta, Gumaste, Montgomery, Mctavis & Grasby, 2005; Monchi, Ko & Strafella, 2006; Mozley, Gur, Mozley & Gu, 2001; Reeves et al, 2005). Loss of dopamine receptors and reductions in dopamine transporter availability (DAT) in the striatum occur in normal aging and are associated with age-related impairments in cognitive function (see Cropley, Fujita, Innis, & Nathan, 2006 for a review). Current research suggests that estrogen can alter dopaminergic function in the ageing human brain. Gardiner et al (2004) used single-photon emission computed tomography (SPECT) to look at possible effects of estrogen on DAT. In their study, postmenopausal women underwent SPECT before and after 4 weeks of estrogen replacement. The results indicated that, relative to baseline levels, estrogen treatment produced an increase in striatal DAT.

Craig et al (2004) compared the growth hormone (GH) response to apomorphine in postmenopausal women who were long-term users of estrogen and women who were estrogen-naïve. Apomorphine is widely used to "challenge" central dopaminergic responsivity (Craig et al, 2004). The results of the study indicated that postmenopausal women who take estrogen over the long-term have enhanced dopaminergic function relative to women who have never taken estrogen. In addition, while loss of striatal dopamine has been found

to be associated with cognitive dysfunction in individuals with PD (Brooks & Piccini, 2006), the results from a large observational study indicate that estrogen protects against the development of dementia in PD (Marder et al, 1998).

In summary, there is a considerable amount of research that demonstrates the ability of estrogen to effect cognition through its influence on the cholinergic, dopaminergic and serotonergic systems. These neurotransmitters have all been shown to be involved in cognitive function and have been implicated in neurodegenerative disorders such as AD and PD, both of which are associated with cognitive dysfunction.

Estrogen and Neuroprotection

Gender differences have been observed during normal aging in areas of the brain relevant for cognition (Moorthy et al, 2005; Xu et al, 2000). In addition, epidemiological and clinical data indicate the existence of a natural sexual dimorphism in the risk, progression and recovery from neurological conditions such as traumatic brain injury, stroke and AD (Czlonkowska, Ciesielska, Gromadzka, & Kurkowska-Jastrezebska, 2005). It has been suggested that certain levels of estrogen are necessary for protection against these diseases (Numakawa et al, 2007).

There is considerable evidence from basic science studies to support a neuroprotective role for estrogen. According to Garcia-Segura, Azcoitia & DonCarlos (2001) one of the most likely explanations for the neuroprotective effects of estrogen is related to its antioxidant properties. *In vitro*, β-amyloid

protein, hydrogen peroxide and glutamate all cause oxidative stress-induced toxicity, which results in cell death (Lee & McEwen, 2001). Oxidative stress is thought to be an imbalance between the endogenous antioxidant system and the generation of free radicals (Jung, Rewal, Perez, Wen & Simpkins, 2004) and has been linked to a variety of neurodegenerative disorders, including AD and PD (Lee & McEwen, 2001). A substantial number of *in vitro* studies have shown that estrogen can protect neurons from the oxidative stress. For example, pretreating cultured neurons with estrogen has been shown to protect them against glutatmate-induced toxicity (Behl, Widmann, Trapp & Holsboer, 1995; Brinton et al, 2000; Nilsen & Brinton, 2002; Sribnick, Ray, Nowak, Li & Banik, 2004; Zhao, Wu & Brinton, 2004). Estrogen also enhances the survival of cultured neurons exposed to toxic levels of β-amyloid (Behl et al, 1995; Brinton et al, 2000; Cordey & Pike, 2005; Xu, Wang, Zhang & Zhang, 2006). Pretreating neurons with estrogen also provides neuroprotection against the neuronal death caused by oxidative stress in cells exposed to hydrogen peroxide (Behl et al, 1995; Biewenga, Cabell & Audesirk, 2005; Brinton et al, 2000; Numakawa et al, 2007; Sawada et al, 1998; Sur et al, 2003). These *in vitro* studies all support the role of estrogen as a neuroprotectant.

Estrogen has also been shown to have neuroprotective effects in *in vivo* models of brain injury. While men and women have the same risk factors for stroke, there are gender differences in the incidence of stroke such that premenopausal women have fewer strokes than same-aged men (Garcia-Segura

et al, 2001). In the rodent model of ischemic stroke, stroke is induced experimentally through permanent occlusion of the middle cerebral artery (MCA), which results in a blockage of blood flow to the cerebral vasculature (Wise, 2005). Using the MCA occlusion paradigm, Alkayed et al (1998) found that young female rats sustained smaller infarcts than same-aged male rats. This gender difference in infarct size disappeared when the young female rats were ovariectomized (Alkayed et al, 1998). In addition, gender differences in infarct size following MCA occlusion do not exist between middle-aged reproductively senescent female rats and same-aged male rats (Alkayed, Murphy, Traystman & Hurn, 2000). These findings suggest a neuroprotective role for estrogen in ischemic stroke. This hypothesis is supported by the results of studies in which the administration of estrogen to ovariectomized rodents has been shown to decrease the volume of the infarct induced by MCA occlusion (Plamondon, Morin & Charron, 2006; Wise, 2006; Won, Kim & Koh, 2006) and to increase the number of surviving hippocampal neurons relative to that observed in untreated animals (Gulinello, Lebesgue, Jover-Mengual, Zukin & Etgen, 2006; Shugrue & Mechenthaler, 2003). Gulinello et al (2006) also examined the functional outcomes of ischemia in ovariectomized rats and the potential cognitive benefits of estrogen. In their study, ovariectomized rats exhibited significant impairments in the performance of tasks designed to assess visual working memory and spatial memory after MCA occlusion. The administration of long-term estrogen to ovariectomized rats prior to MCA occlusion prevented these ischemia-induced

deficits. The administration of estrogen to male and ovariectomized female rats after diffuse traumatic brain injury reduces blood brain permeability and edema formation (O'Connor, Cernak & Vink, 2005) and increases post-injury cerebral blood flow relative to untreated animals (Roof & Hall, 2000).

It is likely that part of the neuroprotective effect of estrogen that has been observed in animal models of brain injury is due to its ability to modulate the expression of several of the proteins that control programmed (apoptotic) cell death. Proteins such as Bcl-2 and Bcl-xl inhibit cell death while Bax, Bad and Bid are proteins that promote cell death (Lee & McEwen, 2001). In animal models of ischemic stroke and traumatic brain injury, estrogen prevents the injury-induced down-regulation of bcl-2 (Soustiel, Palzur, Nevo, Thaler & Vlodavsky, 2005; Wise, 2006; Won et al, 2006) and prevents the injury-induced upregulation of Bax (Won et al, 2006). These studies suggest that estrogen may protect against cell death by altering the balance between the factors that influence cell viability and those that influence cell death (Wise, 2006; Won et al, 2006).

Advances in brain imaging technology have allowed researchers to study the protective effects of estrogen in the human brain. A number of imaging studies indicate that total brain volume and the volume of white and grey matter in the brain decline with normal aging (Brickman, Habeck, Zarahn, Flynn & Stern, 2007; Raz et al, 1997; Salat, Kaye, & Janowsky, 1999). Age-associated reductions in prefrontal and temporal white matter have been associated with age-related declines in performance on tasks of executive function and memory

(Brickman et al, 2006) and hippocampal volumes in healthy older individuals predict declines in verbal memory with aging (Jagust et al, 2006). The rate of total brain atrophy predicts conversion from a clinical diagnosis of mild cognitive impairment (MCI) to AD (Jack et al, 2005) and correlates with the rate of change in MMSE scores in individuals with AD (Fox, Scahill, Crum & Rossor, 1999). Imaging studies that have looked at estrogen and brain structure in postmenopausal women report a beneficial effect of estrogen on brain structure. Postmenopausal women who take estrogen have been found to have significantly larger hippocampal volumes (Eberling et al, 2003; Lord, Buss, Lupien & Pruessner, in press), greater total grey matter volumes (Erickson et al, 2007; Boccardi et al, 2006; Ghidoni et al, 2006) and white matter volumes (Ha, Xu & Janowsky, in press) and to experience a reduced age-related shrinkage of the PFC (Raz, Rodrigue, Kennedy & Acker, 2004) relative to women who do not use ERT. Ghidoni et al (2006) also report that, in addition to having greater grey matter volumes than never users of estrogen, women who had used estrogen also performed better than the nonusers on measures of verbal memory, attention and executive function.

Regional cerebral blood flow (rCBF) measures the rate at which nutrients are delivered to brain tissue and is considered a measure of brain activity (Bentourkia et al, 2000). Reductions in rCBF have been observed in the prefrontal, temporal and parietal cortices of individuals with AD that correlate with scores on the MMSE (Edison et al, 2006; Jagust, Eberling, Reed, Mathis & Budinger,

1997; Nagahama et al, 2003) as well as scores on measures of verbal memory and fluency (Edison et al, 2006). Reductions in rCBF that are independent from brain atrophy occur with normal aging (Bentourkia et al, 2000) and have been observed in the prefrontal cortex of women experiencing moderate to severe climacteric symptoms relative to women not experiencing symptoms (Abe et al, 2006). Greene (2000) examined the effect of estrogen on rCBF in healthy postmenopausal women who were experiencing hot flashes. Women underwent rCBF measurement at baseline and after 6 weeks of estrogen replacement. At baseline, women showed patterns of rCBF comparable to those seen in patients with AD. After six weeks of estrogen treatment, there was a significant improvement in rCBF that was most noticeable in the temporal and parietal regions. Greene (2000) concluded that the results of the study suggest that estrogen's effects on rCBF could represent the link between the hypoestrogenic state of menopause and neurodegenerative diseases such as AD. Maki and Resnick (2000) found that postmenopausal women who took estrogen showed greater longitudinal increases in rCBF, relative to women who did not use estrogen, in a number of brain regions including the hippocampus and temporal lobe. Women who used estrogen also had higher memory scores than women who were nonusers. Maki and Resnick (2000) stated that their finding of differences in rCBF in areas of the brain believed to underlie memory suggests that these are the neural substrates responsible for the enhanced memory performance observed in the women using estrogen.

The regional cerebral metabolic rate for glucose (rCMRGlc) is another measure of brain activity that is related to glucose consumption in neurons (Bentourkia et al, 2000). Reductions occur in the rCMRGlc with normal aging (Bentourkia et al, 2000) and individuals with AD exhibit significant reductions in glucose metabolism, relative to healthy controls, in the parietal, temporal, occipital, frontal and posterior cingulate cortices (Alexander, Chen, Pietrini, Rapoport & Reiman, 2002). Decreases in glucose metabolism have been shown to correlate with declines in scores on measures of global cognitive function (Alexander et al, 2002; Edison et al, 2006; Jagust et al, 2006) and verbal memory in individuals with AD (Edison et al, 2006) and may be useful in predicting conversion from MCI to AD (Chetelat et al, 2003) as well as for monitoring disease progression (Alexander et al, 2002; Hirono, Hashimoto, Ishii, Kazui & Mori, 2004). With regard to estrogen's effects on rCMRGlc, Schonknecht et al (2003) found a significant positive correlation between cerebral spinal fluid levels of estrogen and hippocampal rCMRGlc in postmenopausal women with AD. Rasgon et al (2005) recently reported that while no baseline differences in rCMRGlc were evident between women who took estrogen and women who did not take estrogen, women who did not take estrogen showed a significant decline in rCMRGlc in the posterior cingulate cortex at the two year follow-up. In a similar study, Rasgon et al (2001) found that women who used estrogen had a significantly increased rCMRGlc in the lateral temporal region at the two year follow-up while women who did not use estrogen and men showed no metabolic

changes in this region. Eberling et al (2003) examined the effects of estrogen on rCMRGlc in a group of women who were either taking estrogen or tamoxifen or were taking neither. Given that tamoxifen has been shown to have estrogen antagonistic effects in some brain regions (Eberling et al, 2003), it was hypothesized that tamoxifen would potentiate the decrease in estrogen that occurs with menopause, making changes in cognitive function and brain metabolism more apparent. Results of the study showed that women taking tamoxifen and women who were not taking estrogen also had significant reductions in rCMRGlc relative to women taking estrogen. This was most evident in frontal regions.

In summary, there is considerable evidence that supports the idea that estrogen exerts its neuroprotective effect on cognitive function by altering and/or maintaining the structure and physiology of areas of the brain known to be essential for cognition, such as the neocortex and hippocampus. The next section will examine the evidence supporting a role for estrogen in cognition at the behavioral level.

Estrogen and Cognition

Cognitive Function and the Menstrual Cycle

In younger women, studies of the effects of estrogen on cognition have tended to focus on what are considered sexually dimorphic abilities (Maki, Rich & Rosenbaum, 2002). According to Sherwin (1994), this approach to studying the relationship between hormones and cognition is based on the assumption that

sex hormones influence abilities whose dimorphisms are presumed to be the result of the differential exposure of men and women to sex hormones during the prenatal period.

With regards to sexually dimorphic abilities, the nonhuman animal literature suggests that male members of the species have an advantage over female members on tasks that require spatial ability (Lacreuse, Verreault & Herndon, 2001). The same appears to be true for humans in that it has generally been found that men tend to do better than women on tasks requiring spatial ability such as mental rotation (the ability to rotate figures in the mind) and throwing accuracy (the ability to guide or intercept projectiles; Kimura, 2004). In addition, men also appear to do better on tasks involving mathematical reasoning ability (Kimura, 2004). Women, on the other hand, tend to do better than men on tasks requiring verbal ability such as verbal memory and fluency, and on tasks requiring perceptual speed and accuracy and fine motor skills (Kimura, 2002). Women also do better on tasks of object location memory (Kimura, 2004).

Studies of the rodent estrus cycle and the nonhuman primate menstrual cycle, indicate that females of these species perform better on spatial tasks at points during their respective cycles when estrogen levels are low relative to times when estrogen levels are high (Galea, Kavaliers, Ossenkopp & Hampson, 1995; Lacreuse et al, 2001; Warren & Juraska, 2000).

A number of studies have compared women's performance on a variety of sexually dimorphic tasks at points during the menstrual cycle when estrogen and progesterone levels vary. Hampson & Kimura (1988) compared women's performance during the menstrual (low estrogen and progesterone) and midluteal (high estrogen and progesterone) phases of the menstrual cycle on a task of spatial skills (the Rod and Frame Test), which favors male abilities, and on several tasks of speeded manual coordination (finger-tapping, the Purdue Pegboard and the Manual Sequence Box), which favor female abilities. The results of the study showed that, consistent with the findings from the animal studies of spatial abilities mentioned above, women had poorer performance on the Rod and Frame Test during the midluteal (high estrogen) phase of the cycle than during the menstrual (low estrogen phase of the cycle). In contrast, performance on the speeded manual tasks improved during the midluteal (high estrogen phase of the cycle). No differences were noted in mood between the two phases of the cycle leading Hampson and Kimura (1988) to conclude that their results were supportive of the idea that sex differences on the tasks included in their study might be due to the influence of hormones.

Hampson (1990a) reported similar findings with regard to the Rod and Frame Test and speeded manual coordination in her study. In addition, Hampson (1990a) reported that women in her study performed significantly better on a task of speeded articulation, which favors females, during the midluteal versus menstrual phase of their cycles. A trend for better performance

on measures of verbal fluency and perceptual speed at the midluteal phase was also observed.

Other studies have also reported that women tend to perform better on tasks that require abilities that favor females, such as verbal fluency and the Grooved Pegboard, during phases of the menstrual cycle when estrogen and progesterone are high versus phases when the levels of these hormones are low (Hampson, 1990b; Maki et al, 2002). The reverse pattern of better performance during phases of low versus high levels of estrogen and progesterone has also been observed in other studies of menstrual cycle effects on tasks, such as the Mental Rotations Test and the Rod and Frame Test, that require skills that favor males (Hampson, 1990b; Hausmann, Slabbekoorn, Van Goozen, Cohen-Kettenis & Gunturkun, 2000; Maki et al, 2002; Phillips & Silverman, 1997). Although levels of estrogen and progesterone both fluctuate across the menstrual cycle, there is evidence to suggest that performance differences between the phases of the menstrual cycle on cognitive tasks, especially those that require spatial abilities, are more likely due to variations in estrogen versus progesterone production over the menstrual cycle. For example, Hampson (1990b) found that women's performance on measures of articulation and manual speed significantly improved during the preovulatory phase of the menstrual cycle when secretion of estrogen but not progesterone is greatly increased. Women performed less well on measures of spatial ability during the preovulatory phase. In addition, Hausmann et al (2000) found that women's performance on the Mental Rotations

Test was significantly negatively correlated with levels of estrogen while no correlation was found between levels of progesterone and spatial ability.

Researchers have also looked at the impact of menstrual cycle phase on other aspects of cognition that are considered to be more gender neutral. Phillips and Sherwin (1992a) compared women's performance on a battery of memory tests during the menstrual and luteal phases of the menstrual cycle. Plasma hormone levels were measured to confirm cycle phases. Women completed the logical memory, visual reproduction, paired associate learning and digit span subtests from the Wechsler Memory Scale (WMS). Two forms of the WMS were used in the study with half the subjects receiving Form I first and half receiving Form II. A measure of mood was also included. Results showed that scores on the delayed recall of the visual reproduction subtest were significantly lower in the menstrual phase compared to the midluteal phase. No significant differences were observed between cycle phases for the other WMS subtests. However, plasma estrogen levels were significantly positively correlated with scores on the paired-associates subtest during the midluteal phase of the menstrual cycle.

Maki et al (2002) also reported no differences in performance on a measure of verbal memory between phases of the menstrual cycle. They did observe an effect of menstrual cycle phase on a task of verbal implicit memory (category exemplar generation test) such that priming was superior during the midluteal phase of the cycle (high estrogen) in comparison to the early follicular (low estrogen). Maki et al (2002) concluded that their findings suggest that

estrogen may be capable of modulating neurobiological substrates of memory other than the hippocampus.

Rosenberg and Park (2002) compared performance on a verbal working memory task on days 0 (first day of the menstrual period), 7, 14 and 21 of the menstrual cycle in two groups of young women. The first group (natural cycle group) consisted of women with normal 28-day cycles who had all shown a normal postovulatory rise in basal body temperature (BBT) in the month prior to testing. The second group (control group) consisted of women who had been taking non-tricyclic birth control pills for at least 3 months. During the testing month, women in the natural cycle group recorded daily BBTs. All women showed a postovulatory rise in BBT midcycle. Results of the study indicated that women in the control group, whose estrogen and progesterone levels are steady because of the birth control pill, showed no significant differences in performance on the working memory task across test sessions. Naturally cycling women showed a pattern of significantly better performance on the working memory tasks midcycle, when estrogen levels are high, compared to the beginning and end of their cycles.

In conjunction with the studies reporting better performance on measures of verbal fluency during the high estrogen phase of the menstrual cycle, Rosenberg and Park's (2002) results suggest that fluctuations in hormones during the menstrual cycle can impact tasks associated with frontal lobe functions.

It is important to note that a number of studies have not found differences in cognitive performance between the phases of the menstrual cycle (see Epting & Overman, 1998). Sherwin (2003) has suggested that some of the inconsistencies in the menstrual cycle literature are a reflection of failures to confirm the phases of the cycle through the measurement of hormone levels as well as the use of sample sizes that are too small and the use of inappropriate tasks or phases of the cycle to test the hypotheses in question. Resnick, Perry, Parry, Mostofi and Udell (1998) also suggest that inconsistencies in the findings may also result from the fact that many studies fail to consider the type and intensity of premenstrual symptoms women may or may not experience.

Although there is evidence to suggest that small changes in cognitive function exist between different phases of the menstrual cycle as a result of fluctuations in hormones, it has been suggested that hormone levels are adequate throughout the cycle to preserve the integrity of most cognitive functions and are unlikely to effect their day-to-day functioning (Resnick et al, 1998; Sherwin, 2003). This is supported by the finding that female pilots showed no differences in performance on a flight simulator task between different phases of their menstrual cycles (Mumenthaler, O'Hara, Taylor, Friedman & Yesavage, 2001).

Cognitive Function and Surgical Menopause

Because the ovaries provide 95% of the estrogen in a premenopausal woman's body (Hogervorst, Williams, Budge, Riedel & Rolles, 2000), premenopausal women who undergo bilateral oopherectomy (surgical removal

of both ovaries) experience a rapid decrease in endogenous estrogen and an early "surgical" menopause. Thus, surgical menopause provides an excellent opportunity to examine the impact of estrogen on cognition.

Farrag, Khedr, Abdel-Aleem and Rageh (2002) conducted a study to look at the effect of estrogen deficiency on cognitive function in surgically menopausal women. They compared the pre- and post-surgery performance on a number of cognitive tasks of women who required hysterectomy and bilateral oopherectomy for benign conditions (for example, uterine bleeding and fibroids) with that of control subjects who were matched for age, parity (number of pregnancies), education, weight and height. Subjects completed the Mini Mental Status Exam as well as the digit span, mental control, logical memory, associate learning and visual reproduction subtests from the WMS preoperatively and 3 and 6 months postoperatively. P300 event-related potentials (ERP) were also completed for each subject. ERPs were elicited with an auditory discrimination task. The P300 component of the ERP is considered a physiological index of the duration of stimulus processing (Kok, 2001) and is significantly negatively correlated with neuropsychological performance in normal adults (Walhovd & Fjell, 2002).

Preoperatively, no significant differences were found between test scores of the patients and controls and mean plasma estrogen levels of the surgical patients did not differ from those of the controls. A significant drop in serum estrogen level was observed for the surgery group at both the 3- and 6-month

postoperative assessments in comparison to the preoperative assessment. No such changes were noted for the control subjects. In terms of cognitive testing, a significant decline in the MMSE scores, especially orientation, attention and calculation, was found in the surgery group at 3 and 6 months postoperatively. A significant decline in all WMS subtests at 6 months postoperative was found for the surgery group. A significant prolongation of P300 latency at 3 and 6 months postoperative was also noted for the surgery group. No differences were found on the cognitive measures or P300 latency at the different test times for the controls. In addition, surgery patients who had greater than a 50% decline in serum estrogen had a greater decline in MMSE, digit span, logical memory and associate learning in comparison to patients who had less than a 50% decline in serum estrogen levels. P300 latency was significantly negatively correlated with serum estrogen levels in the surgery group. Farrag et al. (2002) concluded that estrogen deficiency following surgical menopause has a negative impact on many aspects of cognition and that the greater the degree of estrogen decline, the greater the degree of drop in cognitive functioning.

Sherwin (1988) looked at women's scores on the digit span subtest from the WMS, a paragraph recall test, an abstract reasoning task and a measure of response speed before and after they underwent surgical menopause. Unlike Farrag et al. (2002), the fifty women in Sherwin's (1988) study randomly received postoperatively an estrogen-androgen combined preparation, estrogen alone, androgen alone, or a placebo for three months. For the fourth month, all subjects

received placebo after which they were randomly assigned to a different treatment group for three months. At each treatment phase, women receiving hormone replacement scored higher on the four cognitive measures than women receiving placebo. In addition, the scores of women who had received hormones were significantly lower at the end of the placebo month than they had been during either of the treatment phases. Sherwin (1988) concluded that her results suggest that ovarian hormones may be critical for the maintenance of short- and long-term memory and other higher-order cognitive functions.

In a subsequent study, Phillips and Sherwin (1992b) looked at visual and verbal memory in women before and two months after surgical menopause. Postoperatively, women were randomly assigned to receive either estrogen or placebo. No significant differences were noted between the groups preoperatively with regards to memory or mood scores or plasma estrogen levels. Postoperatively, the estrogen levels of the estrogen replacement group exceeded their preoperative levels as well as those of the placebo group. In terms of memory measures, women who received estrogen postoperatively maintained their scores on a paired associates test while the scores of women in the placebo group declined significantly from pre- to post-operative. The estrogen group also showed a significant increase in immediate paragraph recall while the placebo group maintained their baseline scores. No significant differences were found for immediate or delayed recall of visual information. Memory and mood scores

were not correlated. Phillips and Sherwin (1992b) concluded that estrogen selectively affects verbal memory.

The previously discussed studies have looked at the impact of estrogen on cognitive performance in women shortly after undergoing surgical menopause. Verghese et al. (2000) compared cognitive performance in a group of healthy older women who had received estrogen after surgical menopause with an age and education matched group of surgically menopausal women who had never taken estrogen. Unlike the previous studies, testing was conducted many years after the women's surgery, with the average age of both groups at the time of testing being 75 years. Subjects who had received estrogen following surgical menopause performed better than subjects who had never received estrogen on two measures of verbal memory. Verghese et al (2000) stated that their findings suggest that estrogen replacement therapy (ERT) started soon after surgical menopause may result in long-term improved cognitive performance in women.

In summary, the results of the studies on surgical menopause support a role for estrogen in cognition, with improvements in memory with ERT being one of the most consistent findings.

Cognitive Function and Ovarian Suppression with Gonadotropin Releasing Hormone Agonists (GnRH-a)

Further support for estrogen's role in cognition comes from studies that have looked at the effect of gonadotropin releasing hormones agonists (GnRH-a) on cognitive functioning in premenopausal women. Leuprolide acetate depot

(LAD) is a GnRH-a that is frequently given to women to treat benign conditions such as endometriosis (Olive & Pritts, 2001) and to shrink uterine fibroids prior to their surgical removal (Lethaby, Vollenhoven & Sowter, 2002). Chronic administration of LAD, like the removal of the ovaries, results in a rapid depletion of estrogen (Lethaby et al, 2002).

Women receiving LAD have been found to report significantly more memory difficulties during treatment than prior to and after cessation of treatment (Newton, Slota, Yuzpe & Tummon, 1996). These memory complaints appear to be independent of changes in mood and the somatic side-effects of treatment (Newton et al, 1996).

One possible explanation for these perceived changes in memory is that they are the result of the rapid depletion of estrogen that occurs with GnRH-a treatment (Newton et al, 1996). The findings from studies that have examined the effects of GnRH-a treatment on formal measures of memory appear to support this idea. Sherwin and Tulandi (1996) tested premenopausal women before treatment, after 12 weeks of treatment with LAD and again after 8 weeks of treatment with LAD plus estrogen or LAD plus placebo. At each test session, subjects completed a number of verbal and visual subtests from the Wechsler Memory Scales-Revised (WMS-R). Hormonal levels and memory test scores did not differ between the groups at pretreatment test time. However, after 12 weeks of treatment with LAD, verbal memory test scores decreased significantly in comparison to pretreatment for both groups. The decreases in scores on these

measures reversed for subjects who received LAD plus estrogen in the second treatment phase but remained depressed for the LAD plus placebo group. No significant differences were observed in scores on the measures of visual memory at any of the tests time in either group. Sherwin and Tulandi (1996) concluded that their findings, taken together with the findings from previous studies on surgical menopause, suggest that estrogen is critically important for the maintenance of verbal memory.

Palomba et al (2004) also looked at memory functioning in women receiving LAD treatment with or without the addition of Raloxifene, a selective estrogen receptor modulator (SERM), for uterine fibroids. In their study women received LAD alone, LAD with Raloxifene, or no treatment. Raloxifene was administered to determine whether it might reduce some of the menopausal-like symptoms women undergoing GnRH-a treatment experience. All participants completed measures of memory (WMS) and global cognitive functioning (MMSE) as well as questionnaires assessing mood, anxiety, severity of fibroid-related symptoms and health-related quality of life prior to and following 6 months of treatment. Women who did not receive treatment showed no significant changes in their scores on any of the measures included in the study. However, women who took LAD, regardless of whether or not they also received Raloxifene, showed significant declines in their scores on the WMS and MMSE from pre- to post-treatment. Interestingly, the observed declines in cognitive performance were accompanied by significant improvements in mood,

anxiety levels, perceived health related quality of life and significant reductions in the severity of fibroid-related symptoms. Therefore, it would seem that the significant declines in cognitive performance observed in the LAD treated women in Palomba et al's (2004) study were not the result of a negative impact of mood, anxiety, or health related quality of life on cognition and could, perhaps, be better accounted for by changes in estrogen levels due to the ovarian suppression induced by LAD.

Grigorova, Sherwin and Tulandi (2006) recently examined the effects of ovarian suppression on the performance of prefrontal and executive function tasks in premenopausal women. Test subjects included 25 women who required LAD treatment prior to surgery for endometriosis or for the removal of uterine polyps. 25 women who were matched to test subjects for age, education, general intelligence and socioeconomic status were included as controls. Women who required LAD treatment underwent neuropsychological testing prior to (Visit 1) and 4 weeks after (Visit 2) treatment with LAD. Control women were also tested on two occasions that were 4 weeks apart. Neuropsychological testing included measures of verbal learning and memory, executive function, working memory and attention. Mood was also assessed at both test sessions, as were hormone levels. Following 4 weeks of treatment, estrogen levels dropped to within the postmenopausal range for the women who received LAD. Results of the study showed that, while there was no difference between baseline scores on the working memory measures for treated and untreated women, women who

received LAD had a significant decline in their scores from Visit 1 to Visit 2 while control women showed either no change or a significant improvement in their scores depending on the measure in question. The significant decrease in working memory performance observed in the women treated with LAD remained after controlling for the effects of mood and changes in progesterone levels from Visit 1 and Visit 2 leading Grigorova et al (2006) to conclude that the decline in working memory performance was the result of LAD suppression of estrogen production. In terms of the other neuropsychological measures, all women, regardless of treatment, had higher scores at Visit 2 compared to Visit 1 on measures of verbal memory and learning and executive function. Grigorova et al (2006) stated that this finding was most likely the result of practice effects.

In summary, the results of the studies on the cognitive effects of GnRH-a in premenopausal women are consistent with the findings from the previously discussed surgical menopause studies and indicate that suppression of estrogen production is associated with significant reductions in cognitive function. Together, these two types of studies indicate a significant role for estrogen in maintaining cognitive function in women.

Cognitive Function and Estrogen Replacement in Postmenopausal Women

Given the impact of ERT on cognitive function in surgically menopausal women, researchers have compared the cognitive performance of women who take ERT after normal physiological menopause with that of women who do not take ERT after menopause on a variety of cognitive tasks. Consistent with the

findings from the studies of surgical menopause, a number of observational studies of ERT have reported a positive effect of ERT on cognition. Observational studies compare performance on cognitive measures between women who are past or present users of ERT and women who have never used ERT. Unlike experimental studies, the decision as to whether or not a woman receives ERT in observational studies is that of the woman, with the majority of women choosing to take ERT for non-cognitive reasons (Hogervorst et al, 2000). In these studies, women who choose to take ERT following menopause have been reported to perform better than women who do not take ERT after menopause on measures of verbal memory (Ghidoni et al, 2006; Jacobs et al., 1998; Kampen & Sherwin, 1994; Maki, Zonderman & Resnick, 2001; Resnick, Maki, Golski, Kraut, & Zonderman, 1998: Shaywitz et al, 2003; Stephens, Hamilton & Pachana, 2003), visual memory (Resnick, Metter & Zonderman, 1997; Resnick et al., 1998; Smith, Giordani, Lajiness-O'Neill, & Zubieta, 2001), verbal fluency (Grodstein et al, 2000; Kimura, 1995; Miller, Conney, Rasgon, Fairbanks & Small, 2002; Rice et al, 2000), working memory (Bayer, Lord & Prussner, 2006; Carlson & Sherwin, 1998; Duff & Hampson, 2000; Keenan, Ezzat, Ginsburg & Moore, 2001; Miller et al, 2002) and attention (Ghidoni et al, 2006; Løkkegard et al., 2002; Smith et al., 2001). In addition, the results of observational studies indicate that postmenopausal women who take ERT experience an attenuation in global cognitive decline relative to postmenopausal women who do not take ERT (Carlson et al., 2001; Rice et al., 2000; Matthews et al, 1999; Rasgon et al, 2005;

Steffens et al, 1999) and have a reduced risk of developing dementia (Marder et al, 1998; Mortel & Meyer, 1995; Slooter et al, 1999; Tang et al, 1996; Waring et al, 1999).

However, a substantial number of observational studies have not found performance differences on similar cognitive measures between postmenopausal women taking ERT and postmenopausal women not taking ERT (Alhola, Polo-Kantola, Erkkola & Portin, 2006; Alves de Moraes, Szklo, Knopman & Park, 2001; Barrett-Connor & Kritz-Silverstein, 1993; File, Heard, & Rymer, 2002; Fillenbaum, Kurt, Bekci & Karakas, 2006; Morse & Rice, 2005; O'Hara et al, 2005), and it has been pointed out that observational studies of ERT suffer from a number of biases that can ultimately impact the findings (Barrett-Connor, 1991; Mitchell, 2002; Sherwin, 2003). Self-selection is a significant source of bias in the observational studies of ERT in that women who chose to take ERT tend to be of a higher socioeconomic status, are relatively more educated and tend to live healthier lives (Barrett-Connor, 1991). These variables are all known to be associated with better cognitive functioning in later life (Hendrickx, McEwen & van der Udereaa, 2005; Le Carret et al, 2003; Zhao et al, 2005). The fact that observational studies use data based on a woman's own recall of her ERT use may also be a source of bias in these studies (Mitchell, 2002).

The randomized controlled trial (RCT) is the gold standard of research study designs and, in the case of ERT studies, it is particularly useful in that it reduces the confounds associated with self-selection and recall bias that can

occur in the observational studies (Mitchell, 2002). Some of the RCTs of estrogen and cognitive function in postmenopausal women have reported a positive effect of ERT on cognition relative to placebo. For example, Duka, Tasker and McGowan (2000) examined the effects of three weeks of transdermal ERT on performance on measures of memory and frontal lobe function in postmenopausal women. Women were randomly assigned to receive ERT or placebo via a transdermal patch. Both the women and the experimenter were blind to the treatment condition. All women completed a battery of memory and frontal function tasks prior to and after the 3-week treatment protocol. Estrogen levels were assessed for all women before and after treatment with the results indicating that women receiving ERT had significantly higher levels of estrogen post-treatment. In terms of cognitive functioning, the results of the study showed that women who received 3 weeks of transdermal ERT did significantly better on tasks of visual memory than women who received placebo. No significant differences on tasks of verbal memory or frontal function were observed.

Wolf et al (1999) looked at the effects of 2 weeks of transdermal estrogen treatment on memory performance in postmenopausal women. Women completed cognitive testing at baseline and following the treatment. While there were no overall differences in cognition between women receiving ERT and women receiving placebo, the results indicated that women who showed the highest treatment-induced levels of estrogen performed better on a test of verbal memory relative to women showing less of an estrogen increase. Wolf et al (1999)

concluded that estrogen selectively enhances verbal memory and stated that it is important to monitor treatment-induced increases in estrogen levels to be able to relate them to changes in cognitive function. Other RCT studies of ERT have reported a beneficial effect of transdermal ERT over placebo on verbal memory in postmenopausal women (Asthana et al, 1999; Krug, Molle, Dodt, Fehm & Born, 2003). Asthana et al (1999) also reported that the improvements in verbal memory observed in their study were significantly positively correlated with plasma levels of estrogen.

As is the case with the observational studies, there are also a number of RCTs of ERT in postmenopausal women that do not report any beneficial effects of ERT on cognitive function (Almeida et al, 2006; Heinrich & Wolf, 2005; Janowsky, Chavez & Orwall, 2000; Polo-Kantola et al, 1998; Schiff, Bulpitt, Wesnes & Rajkumar, 2005; Wolf, Heinrich, Hanstein & Kirschbaum, 2005; Yaffe et al, 2006). Hogervorst et al. (2000) state that the inconsistencies in the findings in this area of ERT research can be explained by specific factors such as study design, subject selection, tests used, lack of statistical power as well as the dosage, type and duration of estrogen treatment. Despite these methodological problems, the authors of a recent review of the experimental and observational studies that have looked at the effects of ERT on various aspects of cognition in non-demented, postmenopausal women, concluded that there is converging evidence to support a positive effect of ERT on the cognitive functioning of postmenopausal women (Zec & Trivedi, 2002).

Although small sample sizes could account for some of the inconsistencies in the findings, the results of the large-scale Women's Health Initiative Memory Study (WHIMS; Shumaker et al, 1998), suggested that, rather than being beneficial to women, ERT has a negative impact on cognition. The WHIMS was an ancillary study to the Women's Health Initiative (WHI; The Women's Health Initiative Study Group, 1998) study designed to test the hypothesis that ERT could reduce the incidence of dementia in women over the age of 65 (Shumaker et al, 1998). The WHIMS recruited women age 65 and older from the WHI who were randomly assigned to estrogen (women who had had hysterectomies), estrogen plus progesterone or placebo. All women completed the Modified Mini-Mental Exam (3MS) at baseline and at annual follow-ups. Women identified as cognitively impaired, as per their 3MS score, received extensive neuropsychological testing and neurologic evaluation. Women suspected of having dementia underwent comprehensive laboratory testing to confirm and classify the dementia. It was originally proposed that women would be followed for 6 years (Shumaker et al, 1998), however the study ended 2 years earlier than anticipated when researchers with the WHI announced that women receiving ERT (estrogen and progesterone) were at an increased risk of heart disease and stroke and breast cancer and that these risks outweighed any benefits of ERT seen in terms reduction in risk of colon cancer or osteoporosis (Writing Group for the Women's Health Initiative Investigators, 2002). The WHIMS estrogen and progesterone therapy trial ended at this time as well, while the estrogen-only

trial continued. Results of the estrogen and progesterone trial indicated that ERT provided no benefit over placebo for global cognitive function, as determined by scores on the 3MS, (Rapp et al, 2003) and there were no differences between women taking ERT and women taking placebo in terms of the incidence of mild cognitive impairment (MCI; Shumaker et al, 2003). In addition, women taking ERT had a significantly higher risk of developing probable dementia compared to women given placebo (Shumaker et al, 2003). In 2004, the estrogen-only trial of the WHIMS was stopped because an increased risk of stroke was observed in the ERT group relative to the placebo group (Craig, Maki & Murphy, 2005). Unlike the findings from the estrogen and progesterone trial, the results of the estrogen-only trial showed that women receiving ERT (estrogen only) did not have a significantly higher risk of probable dementia than women taking placebo (Shumaker et al, 2004). As was the case with the estrogen and progesterone trial, no significant difference in the risk of MCI was observed between women taking ERT and women taking placebo.

Recently, Resnick et al (2006) released the results of the Women's Health Initiative Study of Cognitive Aging (WHISCA), the largest longitudinal RCT of the effects of ERT, in this case estrogen plus progesterone, on measures of memory and other cognitive functions. The WHISCA was an ancillary study to the WHI and WHIMS designed to examine the effects of ERT on the rate of age-related changes in cognition and affect in postmenopausal women over the age of 65 (Resnick et al, 2004). Women were randomly assigned to receive estrogen

and progesterone or placebo as per the WHI study. Resnick et al (2006) noted that women in their study had been receiving treatment for 1.1 to 5.6 years as part of the WHI study before the start of the WHISCA trial and, as a result, pretreatment measurements of specific cognitive abilities were not available. In the study, 92.2% of the women in the ERT condition and 92.7% of women in the placebo condition completed 2 cognitive assessments. 42.2% of the women in the ERT condition and 44.1% of the women in the placebo condition were able to complete a third assessment before the WHI study termination in 2002. At each assessment, women completed measures of verbal and visual memory, attention and working memory, spatial rotational ability, fine motor speed and affect. No differences in affect were noted between treatment conditions throughout the duration of the study. Results of the WHISCA suggested that long-term (on average 4 to 5 years) treatment with estrogen and progesterone had a negative impact on verbal learning and memory but had a positive impact on visual memory as indicated by a nonsignificant trend for women in the ERT group to show more improvement, relative to women in the placebo group, over time on a measure of visual memory. Resnick et al (2006) concluded that their results suggest that estrogen, in this case combined with progesterone, may have differential effects on cognition and that results of the estrogen-only trial of the WHISCA, which have yet to be released, would provide additional insight into the effects of estrogen on specific aspects of cognition.

Although the results of the WHIMS and WHISCA studies seemed to indicate either no or a negative impact of estrogen on cognition, researchers quickly turned to finding reasons to explain the discrepancies between the results of the WHIMS and what had been hypothesized based on the findings from basic science and previous studies of ERT and cognition.

Potential confounds in the WHIMS trials have been pointed out by a number of researchers. For example, Craig et al (2005) note that 55% of the women participating in the WHIMS trials were noncompliant with treatment at some point in the study. Hypertension, a risk factor for cardiovascular and cerebrovascular disease, which could affect cognition, was also noted to be more prevalent in each of the ERT groups relative to the placebo groups (Craig et al, 2005). Craig et al (2005) also noted that the WHIMS researchers failed to assess apolipoprotein E (APOE) subtype, which has relevance, given that it has been shown that estrogen either has no effect on cognitive performance (Burkhardt et al, 2004) or increases cognitive impairment in women who are positive for APOE ε4 (Yaffe, Haan, Byers, Tangen & Kuller, 2000).

The selection of medroxyprogesterone acetate (MPA) as the progestin to administer in conjunction with estrogen in the WHIMS has been suggested as a contributing factor to the negative findings reported in the estrogen and progestin arm of the WHIMs (Brinton, 2004). Progestins are given to women receiving ERT to prevent endometrial hyperplasia (Rice, Graves, McCurry & Larson, 1997). *In vitro* research has shown that while progesterone and 19-

norprogesterone, alone or in combination with estrogen, protect hippocampal neurons against glutamate toxicity, MPA fails to do so, and when given with estrogen, antagonizes estrogen's neuroprotective effects (Nilsen & Brinton, 2002). In addition, progesterone and 19-norprogesterone, alone and in combination with estrogen, increase expression of the antiapoptotic protein Bcl-2 while MPA fails to do so and blocks estrogen-induced increases in Bcl-2 (Nilsen & Brinton, 2002). It has also been suggested that administering estrogen and progestin in a cyclic manner, as was the case in a number of the observational studies of ERT reporting positive findings (Henderson, 2006), versus the daily combined formulation used in the WHIMS may result in progestin being less likely to attenuate any of the physiologic benefits of estrogen (North American Menopause Society, 2003).

Although the findings regarding progestins do have relevance for the effective use of HRT in the maintenance of cognitive function during aging, they do not account for the failure of the unopposed estrogen trial of the WHIMS to report a positive impact of ERT on cognition. It has been suggested that the type of estrogen and the route through which it was administered to women in the WHIMS may have been a contributing factor (Gleason, Carlsson, Johnson, Atwood & Asthana, 2004). Prior to menopause, estradiol, the most potent naturally occurring estrogen, is synthesized by the ovaries and is the main estrogen available in the female body (Coelingh Bennink, 2004). With menopause, estradiol levels drop precipitously and the main estrogen in the

body is estrone, a less potent form of estrogen that is synthesized in adipose tissue from andosterone (Coelingh Bennick, 2004). Although there is considerable evidence from the basic sciences to suggest that estradiol has many beneficial neuroprotective effects, more information is needed with regard to the effects of estrone (Gleason et al, 2004). In the WHIMS, women were given daily conjugated equine estrogen (CEE; Shumaker at al, 1998), which consists mostly of estrone (Bennink, 2004). The levels of estradiol obtained with ERTs that consist of estradiol are much higher than those obtained with ERTs containing CEE (Gleason et al, 2004). The relevance of this finding is that, as previously discussed, a number of studies have reported a positive correlation between plasma levels of estradiol and performance on a variety of cognitive measures. This difference in the ability of the two forms of ERT to affect estradiol levels may also contribute to Gleason et al's (2004) finding that the majority of clinical trials reporting beneficial effects of ERT on cognition used a form of ERT containing estradiol. In their analysis of the clinical ERT trials, Gleason et al (2004) found that 79% of studies using estradiol for ERT reported beneficial effects of ER on cognition compared to 55% of studies using CEE. In a recent study, Gleason et al (2006) reported that women who had or were taking CEE performed significantly worse on a measure of verbal memory than women who had or were taking estradiol or women who were hormone-naïve. Route of administration may also be relevant in determining the efficacy of ERT. Women in the WHIMS were administered oral ERT (Shumaker et al, 2003). However,

current research suggests that there may be more effective means of administering ERT. Estrogens are well absorbed through the skin, and transdermal ERT provides slow, sustained release of estrogens and more constant blood levels than those that are observed with oral administration (Coelingh Bennick, 2004). While oral estrogens undergo extensive metabolism in the liver resulting in an estrone to estradiol ratio of approximately 5:1 to 7:1, transdermal estrogen bypasses the liver, resulting in a 1:1 estradiol to estrone ratio that is similar to that which is seen in premenopausal women (Gleason et al, 2004). In addition, while oral estrogens have been found to increase levels of C-reactive protein (CRP) in postmenopausal women, transdermal estrogens have no effect on CRP levels (Eilersten et al, 2005; Lacut et al, 2003; Vongpatanasin et al, 2003). CRP is a nonspecific marker of inflammation that is predictive of atherothrombotic events, including stroke (Hirschfield & Pepys, 2003). In a recent review of studies that have examined the effects of CRP levels on cognitive functioning, Kuo et al (2005) reported that the results of these studies indicate that high concentrations of CRP are predictive of cognitive decline and dementia in healthy, older individuals. In this regard, it is worth noting that, as previously discussed, a number of the smaller RCTs that reported a beneficial effect of ER on cognitive function administered transdermal rather than oral ERT to their participants (Asthana et al, 1999; Duka et al, 2000; Krug et al, 2003; Wolf et al, 1999).

Sherwin (2005) states that, perhaps the most compelling explanation for the WHIMS failure to find a beneficial effect of estrogen on cognition is that women who participated in the study were too old at the time they were given estrogen for it to provide any protection. All women in the WHIMS were over the age of 65 at baseline, with 54% of the women being over age 70 and 18% being over age 75 (Craig et al, 2005). The mean age of women in the WHIMS was approximately 72 years (Sherwin, 2005), putting these women, on average, two decades beyond the age at which women in the observational studies would have started taking ERT in order to alleviate menopausal symptoms.

In keeping with the issue of age and ERT, it has recently been proposed that there is a "critical window" of time around the menopause when estrogen replacement may produce a protective effect on cognitive function in postmenopausal women (Brinton, 2004; Maki, 2006a; Pinkerton & Henderson, 2005; Sherwin, 2006; Sturdee & Maclennan, 2006). This concept of a "critical window" is supported by the findings of a number of studies from the basic sciences as well as observational studies with postmenopausal women. Silva, Mel, Freymuller, Haidar & Baracat (2003) reported that rats who received estrogen 4 days after ovariectomy showed significantly higher synaptic density in the hippocampus relative to control animals, while animals who received estrogen 12 days post-ovariectomy showed no changes in synaptic density relative to controls. In middle-aged rats, estrogen has been shown to significantly improve performance during the acquisition and delay trials of a working

memory task when initiated immediately following ovariectomy but not following a delay of 5 months (Daniel, Hulst & Berbling, 2006). As suggested by Silva et al (2003), these findings indicate that the duration of time between ovariectomy and the start of estrogen replacement is critical in determining whether estrogen produces a physiological response or results in recovery of function.

As with the animal studies, observational studies of ERT in postmenopausal women suggest that early versus late initiation of ERT may be beneficial for cognition (Henderson, Guthrie, Dudley, Burger & Dennerstein, 2003; Kang, Weuve, & Grodstein, 2004; Matthews et al, 1999) and may reduce the risk of developing AD (Henderson, Benke, Green, Cupples & Farrer, 2005; Tang et al, 1996; Zandi et al, 2002). In a RCT of the effects of 10 weeks of transdermal estrogen on cognition in postmenopausal women, Dunkin et al (2005) found that time since menopause was significantly related to the clinical effects of ERT. Women in the study who were given ERT and had the fewest years since menopause experienced the greatest positive change on measures of executive function. The performance of some of the women in the study with the longest times since menopause actually deteriorated over the course of ERT treatment. This pattern of findings is consistent with the results of a recent review of the published RCTs of the effects of ERT on neuropsychological function. In her review, Maki (2005) found that, in general, a beneficial effect of estrogen was observed in performance on measures of memory and attention in younger

postmenopausal women (women less than 65 years of age). In contrast, a review of the randomized controlled trials of women taking estrogen at age 65 or older indicated that estrogen had a negative, if any effect at all, on cognitive function (Maki, 2005). It appears that the findings from both the experimental and observational studies of ERT are consistent with the idea that the timing of the initiation of ER in relation to menopause is important when examining estrogen's effects on cognitive function.

More recently, studies of estrogen have started to focus on its effects in younger postmenopausal women. Aveleyra, Carranza-Lira, Ullo-Aguirre & Ostrosky-Solis (2005) examined the effects of 6 months of ERT on the cognitive functioning of women who were all within 36 months of their last menstrual period. Women received either estrogen or placebo for 6 months and completed measures of attention, memory and executive function at baseline and 6 months after the start of treatment. Subjects were matched on age, education, health status and depressive symptoms. None of the women showed evidence of depression at baseline or posttreatment. The results of the study showed that, in comparison to the women who received placebo, women who took ERT for six months showed significant improvements in their performance on measures of working memory and attention.

Bagger, Tanko, Alexandersen, Qin & Christiansen (2005) recently reported the results of a study in which they examined the effects of two to three years of ERT in the early postmenopausal period on the risk of cognitive impairment 5 to

15 years later. Participants in the study were women who had previously participated in a RCT of ERT and bone loss in postmenopausal women. The average age at entry to the RCT was 54 years and the mean age at follow-up was 65 years. Consistent with the "critical window" theory of ERT, Bagger et al (2005) found that for the women who had received ERT for two to three years during early menopause, the relative risk of cognitive impairment at follow-up was significantly decreased (by 64%) in comparison to women who had never taken ERT. Bagger et al (2005) concluded that a short course of ERT in the early phase of menopause might result in long-term protection against cognitive decline.

In a recent pilot study for the Research into Memory, Brain function and Estrogen Replacement (REMEMBER) study, which will look specifically at the timing of estrogen replacement, MacLennan, Henderson, Paine, Mathias, and Ramsay (2006) reported that women who were early initiators of ERT (i.e., started ERT prior to age 56 for women with a uterus and ovaries or within 5 years of surgical menopause) performed better than late initiators (initiation of estrogen after the previously mentioned times) on the MMSE and better than never users on the Trail Making Test Part A and a measure of verbal fluency. Late initiators performed worse than never users on the MMSE, raising the possibility that, as was observed in the WHIMS, estrogen can actually be detrimental to cognition under certain conditions.

Theories have been put forward to explain why estrogen may be beneficial to cognition when initiated close to menopause but have no effect or be

detrimental to cognition when initiated a number of years after menopause. Sherwin (2005) suggests that neurons may become less sensitive to estrogen after a lengthy absence of exposure to it. Brinton (2004) concurs and states "treating women with estrogen after 2 decades of estrogen deficiency, with the expectation of therapeutic benefit, assumes that the brain hasn't undergone some adaptation to this deficiency (p.417)". There is some evidence from rodent studies to support this theory. Adams, Shah, Janssen & Morrison (2001) looked at the effects of estrogen on hippocampal dendritic spine density in the CA 1 region of the hippocampus of young and aged ovariectomized female rats. Consistent with findings from previously discussed studies (Gould et al, 1990; Woolley & McEwen, 1992), Adams et al (2001) found that young animals treated with estrogen showed a significant increase in the density of dendritic spines relative to young animals that did not receive estrogen. However, no such differences were observed between the aged estrogen-treated and untreated animals, leaving Adams et al (2001) to conclude that the CA1 spines of aged animals are less responsive to estrogen. Savonenko and Markowska (2003) looked at the effects of aging on cognitive sensitivity to estrogen in middle-aged and old rodents. In middle-aged ovariectomized animals, estrogen was protective against scopolamine-induced impairments. However, estrogen did not ameliorate the effects of scopolamine in old ovariectomized animals, suggesting estrogen's ability to enhance basal forebrain cholinergic function is altered with aging. Jezierski and Sohrabji (2001) compared the regulation of BDNF by estrogen in

ovariectomized young adult and reproductively senescent rats. Both groups of animals received estrogen and the results of the study showed that, while young adult rats responded to estrogen with an increase in BDNF in the olfactory bulb and basal forebrain, the old rats did not. Based on their findings, Jezierski and Sohrabji (2001) hypothesized that changes in hormone synthesis during aging may alter the responsiveness of neurotrophin receptors to estrogen.

To account for the differential effects of estrogen with age, Brinton (2005) offers the "healthy cell bias of estrogen" hypothesis in which estrogen is beneficial if neurons are healthy at time of exposure but can exacerbate dysfunction if neurological health is already compromised. Studies in animals support the "healthy cell bias" theory. In ovariectomized rats, estrogen has been shown to be most effective against β-amyloid-induced degeneration of hippocampal neurons when administered before or simultaneously with β-amyloid exposure (Chen, Nilsen, & Brinton, 2006). When administered following exposure to β-amyloid, estrogen was ineffective in reversing β-amyloid-induced degeneration and exacerbated neuronal death (Chen et al., 2006). In their study of the effects of estrogen in old ovariectomized rats, Markowska and Savonenko (2002) found that estrogen improved cognitive ability but only in animals in which there was no evidence of premorbid cognitive deficit due to aging. The findings from these animal studies are consistent with the human studies that indicate that estrogen provides no cognitive benefit to women already diagnosed with AD (Hogervorst et al, 2000), and suggest that while estrogen may be able to

prevent or delay neurodegenerative processes, it is ineffective in reversing them. The current guidelines set by the North American Menopause Society (2007) for the use of ERT in postmenopausal women conclude that, based on the available evidence, initiating ERT after age 65 is not recommended for primary prevention of dementia or cognitive decline and ERT does not appear to provide any benefit for the treatment of AD.

In summary, the current evidence suggests that ERT does not have beneficial effects and may actually be detrimental to cognition in women who are well past menopause at the time they initiate ERT. However, there is some evidence to suggest that estrogen may protect against cognitive decline when initiated early in menopause while the brain may still be responsive to estrogen and before any neurodegenerative processes have begun.

Cognition and Endogenous Estrogen Exposure

If, as the findings from the previous section suggest, estrogen can improve aspects of cognition, it seems reasonable to expect that the longer a women is exposed to her own endogenous estrogen, the less of a decline in cognitive functioning she will experience in later life. There is evidence to support this idea. The length of a woman's reproductive period is considered a marker of exposure to endogenous estrogen (Hagemans et al, 2004) and is defined by subtracting age at menarche (first menstruation) from age at menopause (Geerlings et al, 2001; Rasgon et al, 2006; Saltiki et al, 2006). Studies have found that women with a longer reproductive period, implying a greater exposure to

endogenous estrogen, have a reduced risk of developing cataracts (Younan et al, 2002) and glaucoma (Lee, Mitchell, Rochtchina & Healey, 2003) than women with shorter reproductive periods. A longer reproductive period may also reduce the risk of myocardial infarction (Saltiki et al, 2006). In terms of cognition, Rasgon et al (2005) recently reported that length of reproductive period was significantly negatively correlated with risk of impairment in a sample of postmenopausal women. Women with reproductive periods that were longer than 39 years had a significantly reduced risk of cognitive impairment relative to women whose reproductive periods were less than 35 years, suggesting that endogenous estrogen exposure may be protective against cognitive decline. Bagger, Tanko, Alexandersen, Qin and Christriansen (2004) reported similar findings in their study of older postmenopausal women. After controlling for possible confounding variables such as age, education and depression, Bagger et al (2004) found that women with the shortest reproductive periods (less than 32 years) had the worst cognitive performance, as indicated by scores on the Short Blessed Test, while women with the longest reproductive periods (more than 38 years) had the best cognitive performance. However, a longer reproductive period and hence, greater endogenous estrogen exposure, may not be beneficial to all women. While Geerlings et al (2001) found no association between length of reproductive period and dementia in a large sample of postmenopausal women without an APOE ε4 allele, they did find a significant positive association

between length of reproductive period and risk of dementia in women in their sample who had tested positive for at least one APOE ε4 allele.

A number of studies suggest that individual reproductive events such as age at menarche, age at menopause, and parity can also affect a woman's lifetime exposure to endogenous estrogen and influence her risk for cognitive decline. A younger age at menarche has been associated with enhanced cognitive function in postmenopausal women, as measured with the MMSE (Smith et al, 1999) and the Mental Status Questionnaire (Rasgon et al, 2005). In addition, an older age at menarche may increase a woman's risk for developing AD later in life (Paganini-Hill & Henderson, 1994).

The age at which a woman undergoes surgical menopause is significantly correlated to performance on measures of verbal memory, with women obtaining higher scores when surgery occurs at an older age (Nappi et al. 1999). In a longitudinal study of postmenopausal women, McLay, Maki and Lyketos (2003) found that age at menopause was significantly negatively correlated with degree of cognitive decline, such that women who went through menopause at older ages experienced significantly less of a decline in cognition, as evidenced by scores on the MMSE, than women who experienced menopause at an earlier age. McLay et al (2003) stated that their results suggest that age at menopause predicts the rate of cognitive decline in later life. Age at menopause in women with Down's Syndrome, who are at high risk of developing dementia, has been found to correlate significantly with the age at onset of dementia, with Cosgrave,

Tyrrell, McCarron, Gill, & Lawlor (1999) reporting that, in their study, the younger a woman with Down's Syndrome was at menopause, the younger she was at the onset of dementia. Cosgrave et al. (1999) stated that this suggests a possible role for estrogen deficiency in the development of dementia in women with Down's syndrome.

Pregnancy also affects estrogen levels. Although estrogen levels are very high during pregnancy, they drop precipitously after childbirth with decreased levels maintained for as long as a year (Sobow & Kloszewska, 2004). The results of a study by Dorgan et al (1995) suggest that pregnancy also induces long-term alterations in the secretion or metabolism of estrogen. Dorgan et al (1995) looked at the effects of age at menarche and parity on hormone levels in premenopausal women. The results of their study showed that plasma estradiol levels increased with age during the follicular phase of the menstrual cycle in both nulliparous and parous women. However, while nulliparous women also experienced an increase with age of plasma estradiol during the luteal phase and midcycle, parous women experienced a decrease in plasma estradiol with age during these phases. Age at menarche had no effect on hormone levels. Chubak et al (2004) also found that parity was negatively associated with free estradiol levels in their sample of postmenopausal women. The results of these two studies suggest that nulliparous women may have higher lifetime estrogen exposure than parous women.

Studies that have looked at the effect of parity on cognitive function in postmenopausal women have found that nulliparous women experience significantly less cognitive decline with age, as measured with the MMSE, than parous women (McLay et al, 2003). After controlling for the effects of age and educational attainment, Rasgon et al (2005) found that women in their study who had 5 or more children had a significantly increased risk of cognitive impairment than women with fewer than 5 children. In addition, parity has been associated with both a higher risk of developing AD (Ptok, Barkow & Heun, 2002) as well as an earlier age of onset the disease (Sobow, Kutter & Kloszewska, 1999).

Breastfeeding also has an impact on estrogen levels. During lactation, estrogen production is suppressed, so a woman's exposure to endogenous estrogen during this period is negligible (De Kleijn et al, 2002). Duration of breastfeeding is inversely correlated with the risk of developing breast cancer in both premenopausal and postmenopausal women (see Ma, Bernstein, Pike, & Ursin, 2006 for a review) and it is thought that this is the result of the reduction in cumulative exposure to endogenous estrogen that occurs with breastfeeding (Bernstein, 2002). Dvornyk et al (2006) recently reported that breastfeeding is associated with an earlier age of menopause, thus providing another way in which breastfeeding reduces a woman's lifetime exposure to endogenous estrogen. While breastfeeding effects endogenous estrogen exposure, to date no studies have specifically looked at its effect on cognitive function in postmenopausal women.

After menopause, body mass index (BMI) is considered the strongest marker of endogenous estrogen in postmenopausal women (Grodstein, Clarkson & Manson, 2003). Research in postmenopausal women has shown a significant positive association between estradiol levels and BMI, with estradiol levels increasing as BMI increases (Verkasalo, Thomas, Appleby, Davey & Key, 2001). In a recent study of obesity and cognition in women with Down's syndrome, Patel et al (2004) reported that obese, as determined from BMI scores, postmenopausal women with Down's performed significantly better than non-obese postmenopausal women with Down's on several measures of verbal memory and a global measure of cognitive function, the Down Syndrome Mental Status Exam (DSMSE). In premenopausal women with Down's Syndrome, there were no significant differences between obese and non-obese women on any of the cognitive measures. Mean estrogen levels in the obese postmenopausal women were approximately 67% higher than those observed in non-obese women, leaving Patel et al (2004) to conclude that the higher endogenous estrogen levels that occur with obesity in postmenopausal women with Down's syndrome are associated with better cognitive performance. It should be noted that a recent meta-analysis of the available studies that have looked at the relationship between BMI and dementia reported that the majority of studies found that individuals, especially women, with higher BMIs have an increased risk of developing dementia (Gorospe & Dave, 2007). However, all but one of the studies in the meta-analysis controlled for the effects of APOE-ε4 carrier status, a

factor that would be relevant for postmenopausal women, given that ERT has been found to increase cognitive impairment in women who are positive for the APOE-ε4 allele (Yaffe et al, 2000) and that a longer reproductive period, indicative of greater exposure to endogenous estrogen, increases risk for dementia in APOE-ε4carriers (Geerlings et al, 2001). Consistent with this idea, Brubacher, Munsch and Stahelin (2004) reported that the negative effects of weight change on cognition that they observed in their study of older men and women were exacerbated in individuals who were APOE-ε4 carriers relative to non-carriers.

Bone mineral density (BMD) has been suggested as a potential marker for cumulative estrogen exposure (Tan et al, 2005; Yaffe et al., 1999). BMD has been found to correlate with age at menarche, age at menopause, parity and length of the reproductive period (Grainge, Coupland, Cliffe, Chilvers & Hosking, 2001; Hagemans et al, 2004; Ito et al., 1995; Vega, Egea, & Mautalen, 1994). Yaffe et al. (1999) used BMD as a marker of cumulative estrogen exposure and reported that, after adjusting for age, BMD was significantly positively correlated with performance by postmenopausal women who had never taken ERT on measures of general cognitive functioning and attention.

BMD has also been found to correlate with verbal memory impairment in older women, such that, after adjusting for age, the presence of verbal memory impairment increases as BMD decreases (Zhang, Seshadri, Ellison, Heeren & Felson, 2001). In a recent study, Tan et al (2005) reported that the

postmenopausal women in their study whose BMD measurements were in the lowest quartile had more than twice the risk of developing AD than women whose BMDs were in the other three quartiles. A similar relationship existed between BMD and all-cause dementia. No significant relationships were noted between BMD and risk of AD or all-cause dementia in the male subjects in the study, leaving Tan et al (2005) to conclude that a higher level of cumulative estrogen exposure, as determined by BMD, may reduce a woman's risk of developing dementia. It is worth noting that none of the women in Yaffe et al.'s (1999) study had ever taken ERT, and that when Zhang et al. (2001) excluded women who had taken ERT from their analysis, the significant inverse relationship between BMD and verbal memory impairment remained. In Tan et al's (2005) study, the increased risk for AD and all-cause dementia that existed for women with the lowest BMD remained statistically significant after controlling for the effects of ERT. This pattern of results indicates that higher levels of cumulative endogenous estrogen exposure may be associated with a lower risk of cognitive decline in postmenopausal women, regardless of whether or not they have taken ERT.

To summarize, it appears that the cumulative amount of endogenous estrogen a woman is exposed to over her lifetime can influence cognitive function later in life and that variables that are known to influence estrogen levels during a woman's reproductive and postmenopausal life may have positive or negative influences on cognitive functioning in later years.

Cumulative Estrogen Exposure and Cognition

In the previous sections of this paper, evidence has been presented that suggests that the degree of exposure a woman has to endogenous and to exogenous estrogen may be relevant to cognition function postmenopause. It would seem reasonable to suggest then, that research looking into the effects of estrogen on cognition needs to consider both of these variables. The failure of previous studies that have looked at the effect of ERT on cognition to include variables known to influence endogenous estrogen could account for some of the inconsistencies in their findings. There is some evidence to support this. In a recent RCT of the effects of 10 weeks of transdermal ERT on neuropsychological function in postmenopausal women, Dunkin et al (2005) initially found no differences between women who had been given ERT and women receiving placebo. However, further analyses revealed that the clinical response of women in their study to ERT was influenced by the variables of time since menopause and BMI. Dunkin et al (2005) found that women who were closer to menopause at the time of the study demonstrated greater improvement on measures of executive function than women who were further from menopause at the start of ERT. In addition, women with higher BMIs had significantly greater improvement on measures of attention and psychomotor speed than women with lower BMIs. The positive effect of BMI on measures of attention and psychomotor speed was also observed in women in the placebo group,

suggesting, as indicated in previous studies, that higher levels of postmenopausal endogenous estrogen can have a positive effect on cognition.

Rasgon et al (2005) examined the relationship between total length of estrogen exposure and risk of cognitive impairment in 6,604 postmenopausal women between the ages of 65 and 84. Total length of estrogen exposure was defined as the duration of ERT plus the length of the reproductive period (age at menarche subtracted from age at menopause). Cognitive functioning was assessed using the TELE, a telephone interview with established validity for the identification of individuals with dementia (Ragson et al, 2005). The results of the study showed that, after controlling for age and education, women who had the longest total lifetime exposure to estrogen had the least cognitive impairment. A shorter length of reproductive period was found to be significantly associated with an increased risk of cognitive impairment, suggesting the importance of endogenous estrogen. In addition, a significant independent positive effect of ERT use on the risk of cognitive impairment was also observed, with women in the study who had used ERT experiencing a 40% reduction in risk of cognitive impairment relative to women who did had not used ERT. Rasgon et al (2005) suggest that commencing ERT at the start of menopause may result in a more continuous overall lifetime exposure, which may help to protect against cognitive decline.

Smith et al. (1999) developed an index of cumulative estrogen exposure (IEE) that included variables that, as previously discussed, are well recognized to

affect estrogen levels (time on ERT, age at menarche and menopause, parity, postmenopausal weight and time since menopause). In their study, Smith et al (1999) examined the relationship between scores on the IEE and four factors that consisted of statistically related neuropsychological measures. Factor 1 included Digit Span Forward and Digit Span Backward from the WAIS-R, a measure of letter fluency (FAS) and a measure of category fluency (animals). Factor 2 was made up of the total and delay scores of a 10-item wordlist. Factor 3 included the MMSE, the Boston Naming Test and the Test of Orientation and Naming. Finally, Factor 4 consisted of Visual Span Forward and Visual Span Backward from the WMS along with Judgment of Line Orientation and a task that involved copying geometric drawings. The results of the study showed that, after controlling for age and education, scores on the IEE were significantly related to Factor 3. No significant relationships were found between the IEE and the other three factors. The relationship between the IEE and Factor 3 remained significant after controlling for current use of ERT, from which Smith et al (1999) concluded that estrogen exposure across the lifespan has an effect that is unrelated to current use of ERT. Examining the results of a series of post hoc analyses, Smith et al (1999) reported that no individual marker of estrogen exposure, including duration of ERT, showed as consistent a pattern of relationships with cognitive performance as the IEE. Smith et al (1999) concluded that combining the effects of markers of endogenous and exogenous estrogen might be the most beneficial approach

when trying to understand the effects of estrogen on the brain and cognitive functioning.

Given that endogenous and exogenous estrogen appear to have significant independent and interactive effects on cognitive function in postmenopausal women, it seems reasonable that any researcher who is interested in exploring the relationship between ERT and cognition needs to design a study that takes both of these variables into account. As previous studies have suggested, the clinical response to ERT may depend on its interaction with variables known to influence lifetime levels of endogenous estrogen exposure.

CHAPTER 2

Present Study

To date, no study has looked at the relationship between cumulative estrogen exposure and prospective memory in older postmenopausal women. Retrospective memory is memory for information acquired in the past (Huppert & Beardsall, 1993). Einstein & McDaniel (1996) define prospective memory as "memory for actions to be performed in the future". Remembering to return a video, to make your credit card payment or to take medication are all examples of activities that require prospective memory.

Einstein and McDaniel (1990) distinguish between "time-based" and "event-based" prospective memory. They state that event-based prospective memory is triggered by a particular event or cue (for example, remembering to get gas when you see a gas station), whereas time-based prospective memory requires a self-initiated response at a specific time in the absence of any external cues (for example, remembering to take medication at 8pm). Like retrospective memory (Rendell & Thomson, 1999; Stuart-Hamilton, 2000), both forms of prospective memory decline with age (Daum, Gräber, Schugens, & Mayes, 1996; Huppert, Johnson & Nickson, 2000; Mäntylä & Nilsson, 1997; Maylor, 1996; Maylor, Smith, Della Sala & Logie, 2002; Rendell & Thomson, 1999; Uttl, Graf, Miller & Tuokko, 2001), with most studies reporting larger age deficits on time-based prospective memory tasks (Einstein, McDaniel, Richardson, Guynn, & Cunfer, 1995; Park, Hertzog, Kidder, Morrell & Mayhorn, 1997). This is thought

to be due to the higher degree of self-initiation required for time-based tasks (Einstein & McDaniel, 1990; Maylor et al., 2002; Park et al., 1997), and is consistent with the idea that older adults are more disadvantaged on tasks with high self-initiated processing demands (Craik, 1986). Studies have shown that older individuals who are more successful on tasks of time-based prospective memory often use techniques to transform the time-based task into an event-based task (Maylor, 1990).

Prospective memory is of considerable relevance for the older woman, in that deficits in prospective memory greatly influence an individual's ability to live independently (Einstein & McDaniel, 1996: Flannery, Butterbaugh, Rice & Rice, 1997) and that deficits in habitual prospective memory may be associated with poor medication adherence in older individuals (Vedhara et al, 2004). Prospective memory also has important social implications, in that prospective memory failures are often attributed to a person being unreliable (Zeintl, Kliegel, Rast & Zimprich, 2006). In addition, individuals with AD demonstrate significant deficits in prospective memory tasks in comparison to healthy age-matched controls (Maylor et al, 2002), and studies suggest that impairment in prospective memory may be an early indicator of dementia (Duchek, Balota & Cortese, 2005; Huppert & Beardsall, 1993; Huppert et al., 2000; Jones, Livner & Backman, 2006).

There are a number of reasons to expect that greater cumulative estrogen exposure may improve an older woman's prospective memory. Firstly, neuroimaging studies have demonstrated that the PFC is the primary

neuroanatomical substrate for prospective memory (Burgess, Quayle & Frith, 2001; Okuda et al., 1998). As previously discussed, numerous studies in rodents and nonhuman primates have shown that estrogen is capable of modulating neuronal properties as well as altering neurotransmitter levels in the PFC. ERs have been detected in the human PFC (Perlman et al, 2005a; Perlman et al, 2005b), and the results of neuroimaging studies indicate that estrogen is capable of modulating rCBF and brain activation patterns in the PFC of premenopausal (Berman et al., 1997) and postmenopausal women (Joffe et al, 2006; Resnick et al., 1998; Shaywitz et al., 1999; Smith et al, 2006; Stevens, Clark & Prestwood, 2005).

Secondly, hormone replacement studies support the role of estrogen in the prefrontal cortex. Women who take ERT have been found to perform better than untreated women on frontal-mediated tasks such as measures of verbal and spatial working memory (Duff & Hampson, 2000; Keenan et al., 2001), verbal fluency (Kimura, 1995; Grodstein et al., 2000), susceptibility to interference (Wolf & Kirschbaum, 2002) and perseveration (Erickson et al, 2007; Ghidoni et al, 2006; Schmidt et al, 1996).

It has been suggested that the PFC and its circuitry are the prime mediators of estrogen's role in cognition, rather than the hippocampus (Keenan et al, 2001; Krug et al, 2006), and there is some evidence from clinical studies to support this. For example, Krug et al (2006) found that postmenopausal women who received 3 days of transdermal ERT performed significantly better on tasks that involve the PFC (e.g., the Stroop, a digit ordering task and a memory

sequencing task) than women who received placebo, but showed no significant enhancements in performance on hippocampal-dependent tasks relative to placebo-treated women. Krug et al (2006) concluded that short-term administration of estrogen preferentially enhances performance on tasks dependent on the PFC. Joffe et al (2006) also reported a selective effect of estrogen on prefrontal function. In their study, perimenopausal and postmenopausal women between the ages of 40 and 60 were randomly assigned to receive either transdermal estrogen or transdermal placebo for 12 weeks. All women completed measures of verbal and visual learning and memory and executive function (in this case, the number of perseverative errors on the CVLT) at baseline and at the end of treatment. Results of the study indicated that women who received 12 weeks of transdermal estrogen made significantly fewer perseverative errors on a measure of verbal recall than women who received placebo. No significant differences were observed on any of the memory measures. In a subset of women who underwent fMRI, those who received ERT exhibited significantly greater activation than untreated women in several prefrontal regions while completing measures of verbal memory and visual working memory. Joffe et al (2006) concluded that ERT has a selective effect on frontally mediated executive functions. Keenan et al's (2001) finding that postmenopausal women treated with ERT outperform untreated women on measures of attention, inhibition and cognitive set switching but not verbal memory is consistent with this idea. Duff and Hampson (2000), who found that

women in their study who were taking ERT performed better on PFC-dependent measures of working memory, but not on measures of hippocampal-dependent short-term verbal memory, suggest that some of the inconsistency in the ER literature with regard to the effects of ER on verbal memory could be explained by the degree to which the tasks used in the various studies depend upon frontal lobe functions.

It appears that there is considerable evidence to support the hypothesis that cumulative estrogen exposure may be associated with prospective memory. In terms of what is already known about estrogen's impact on prospective memory, there are three hormone replacement studies that have included a measure of prospective memory, although it was not the focus of the studies. In the first of these studies, Resnick et al. (1998) found no difference in scores on the prospective memory component of the Rivermead Behavioural Memory Test (RBMT) between postmenopausal women receiving ERT and untreated women. In a subsequent study, Maki & Resnick (2000) reported that ERT users performed significantly better than nonusers on the prospective memory portions of the RBMT across two time points. Both of these studies had very small sample sizes and Maki & Resnick (2000) stated that, while their findings regarding prospective memory were suggestive, they required replication. More recently, Stephens, Hamilton and Pachana (2003) administered the Rivermead Behavioural Memory Test Extended (RBMT-E) to a group of postmenopausal women between the ages of 40 and 60 (mean age of approximately 52 years) to

determine whether ERT has any effect on everyday memory functioning. The RBMT-E includes measures of prospective memory (Stephens et al, 2003). Results of the study showed that, among the older participants in the study (women who were older than 52), those who used ERT performed significantly better on the prospective memory tasks of the RBMT-E than the women who were nonusers of ERT. No such differences were observed between younger users and nonusers of ERT, suggesting that ERT might protect women against age-related cognitive decline (Stephens et al, 2003).

The previous studies of estrogen and prospective memory focused on the effects of ERT on prospective memory and, as stated before, no study to date has determined whether cumulative estrogen exposure has any bearing on prospective memory in later life. Therefore, the purpose of the present study is to look at whether there is a relationship between the level of cumulative estrogen exposure, as estimated with an index of variables known to influence estrogen levels, and a woman's performance in later life on a task designed to assess prospective memory. In keeping with Rasgon et al's (2005) description of cumulative lifetime exposure to estrogen as the "sum of endogenous exposure throughout the reproductive period and exogenous exposure via use of hormone therapy in menopause" (p.559), cumulative estrogen exposure will be determined with an index of variables known to influence estrogen levels throughout a woman's life. Based on the previously discussed research findings, this index will include age at menarche, age at menopause, postmenopausal BMI,

parity, time since menopause, duration of breastfeeding and duration of ERT. Given that the empirical evidence suggests that time-based tasks of prospective memory are generally more sensitive in older adults (Einstein et al, 1995; Park et al, 1997), the present study will focus on time-based rather than event-based prospective memory. In keeping with the findings from the basic science literature and the clinical studies of endogenous estrogen and ERT, it is hypothesized that in a sample of older postmenopausal women, women with higher levels of cumulative estrogen exposure will perform better on a measure of time-based prospective memory relative to women with lower levels of cumulative estrogen exposure. For comparison purposes, measures of verbal memory will also be included with the expectation that, based on previous findings, women with higher levels of cumulative estrogen exposure will perform better on these measures.

Chapter 3

METHOD

Participants

Participants in this study were women between the ages of 65-74 who voluntarily responded to advertisements placed in local publications and community centers seeking volunteers for a study on memory in older women (see Appendix A). Women were screened over the telephone for study eligibility when they called in response to the advertisement. Women were considered for inclusion if they considered themselves to be in good physical and mental health and were able to provide information with regard to the timing and nature of menopause as well as use of hormone replacement. Exclusionary criteria included self-reported history of hysterectomy without bilateral oopherectomy (as age at hysterectomy in these women would, according to Younan et al (2002), underestimate their age at menopause), central nervous system disease (e.g., Parkinson's, Multiple Sclerosis, clinical history of stroke, epilepsy and other neurological disorders), psychiatric diagnosis, severe cardiac disease (including history of myocardial infarction, coronary bypass surgery or angioplasty), or metastatic cancer. A total of 62 women volunteered for and completed the study. The participant characteristics of the sample are shown in Table 1.

Table 1: Participant characteristics of the sample (N=62)

	Mean	Standard Deviation
Age	69.3	3.2
Education (yrs.)	15.1	2.8
IQ	103.1	15.4
GDS	1.7	2.0
RAND Health	78.4	13.9
RAND Pain	82.6	16.9

Note: IQ = intelligence quotient; GDS = score on the Geriatric Depression Scale; RAND Health = score on the General Health scale of the RAND 36-Item Health Survey 1.0; RAND Pain = score on the Pain scale of the RAND 36-Item Health Survey 1.0. The General Health and Pain scales from the RAND are scored so that high scores indicate better health and less pain.

Measures

Spot-the-Word-Test (STW) from the Speed and Capacity of Language Processing Test (SCOLP; Baddeley, Emslie, & Nimmo-Smith, 1992) – this is a lexical decision task in which subjects are presented with 60 pairs of words. One word in each pair is a real word while the second word is a pseudoword. Subjects are required to identify the real word in each pair. There is no time limit. The Spot-the-Word test is administered as a measure of current intellectual functioning. Possible raw scores range from 0 to 60. Raw scores were converted to IQ estimates as per Crowell, Vanderploeg, Small, Borenstein Grave, & Mortimer (2002). The STW is

included in the test battery to ensure that differences in memory performance are not due to differences in level of intellectual functioning.

Geriatric Depression Scale (GDS; Brink et al., 1982) - this is a self-report measure consisting of 30 yes/no questions that is designed to screen for symptoms of depression in individuals over the age of 65. Possible scores range from 0 to 30 with scores from 11 to 19 considered to be suggestive of mild depressive symptomatology and scores higher than 20 indicating moderate to severe depressive symptomatology (Woodard & Axelrod, 1999). Late-life depression has been shown to affect memory, attention and executive functioning (Elderkin-Thompson, Mintz, Haroon, Lavretsky, & Kumar, 2006; Gallassi, Di Sarro, Morreale, & Amore, 2006; Lockwood, Alexopoulos, Kakuma, & Van Gorp, 2000). In addition, clinically depressed adults have been found to perform significantly worse on a task of time-based prospective memory than nondepressed controls (Rude, Hertel, Jarrold, Covich, & Hedlund, 1999). As a result, the GDS was included in the present study to ensure that participants were not experiencing any symptoms suggestive of depression that could influence their performance on the cognitive measures. Examination of GDS scores indicated that none of the women in the study were experiencing symptoms suggestive of depression at the time of testing.

Logical Memory, (WMS-III, Wechsler, 1997) – this is a measure of short-term verbal memory in which the subject is read two short stories and then asked to repeat the stories as close to verbatim as possible (immediate recall). Subjects are

asked to repeat the stories again 30 minutes later (delayed recall). Logical Memory was selected as a measure of verbal memory because it is the task for which significant positive findings have been reported most consistently for women taking estrogen replacement therapy (Zec & Trivedi, 2002b).

Time-based prospective memory task – this task is based on Martin and Schumann-Hengsteler (2001). Subjects were asked to complete simple pencil and paper arithmetic questions for 20 minutes. For the prospective memory component of the task, subjects were asked to change the color of the pencil they were writing with every 3 minutes (i.e., to a maximum of six times). A jar containing a number of colored pencils was placed to the subject's right and a digital timer with large digits that counted minutes and seconds up from zero was placed to the subject's left so that they could monitor time. For each time the subject changed their pencil within 30 seconds of the target time (e.g., a time between 2 minutes 30 seconds and 3 minutes 30 seconds was considered a correct response for the 3 minute time change), a score of 1 point was given (for a minimum of zero points and a maximum of six). As per Martin and Schumann-Hengsteler (2001), subjects were motivated to perform the prospective memory task by the experimenter telling them that the task was designed to study problem-solving processes and that the change of pencil color would allow the researcher to determine the rate at which they solved problems across the test period. After being told the instructions for the task, subjects were asked to immediately repeat the instructions to ensure that they understood what they

were being asked to do. At the end of the 20 minutes, the experimenter asked any woman who failed to change pencils at all during the 20-minute task the following: "At the beginning of this task I asked you to do something every 3 minutes in addition to completing the arithmetic questions. Can you tell me what that was?" As per, Jones et al (2006), failure to recall the task instruction with this cue was seen to reflect failure of the retrospective component of the prospective memory task as the prospective cue was provided in the question. Only one woman in the sample failed to change her pencil at least once during the task. When questioned at the conclusion of the task she was able to recall the task instructions suggesting that her lack of pencil change was due to the prospective memory component of the task.

Digit Span Forward, WMS-III (Wechsler, 1997) – this is a measure of attention/freedom from distractibility (Lezak, 1995) in which participants are asked to repeat sequences of digits of progressively increasing length. The score is the number of digits repeated correctly. Measures of freedom from distractibility, including digit span, have been included in a number of experimental and observational hormone replacement studies and have been found to be generally insensitive to the effects of estrogen replacement (Zec & Trivedi, 2002b). Thus, digit span forward was included in the present study as a control measure. Digit span backward was not included as it is a measure of working memory, which may be responsive to estrogen (Duff & Hampson, 2000).

RAND 36-Item Health Survey 1.0 (Hays, Sherbourne, & Mazel, 1993) –this is one of the most widely used health-related quality of life survey instruments available (Hays & Morales, 2001). It is a self-report measure that consists of 36 items that assess eight areas of health: physical functioning, role limitations caused by physical health problems, role limitations caused by emotional problems, social functioning, emotional well-being, energy/fatigue, pain, and general health perceptions. Scores on all eight subscales range from 0 to 100, with higher scores indicating better health or function. Scoring is done so that, regardless of the health area in question, higher scores indicate better functioning. Given that subjective ratings of health have been found to correlate with performance on tasks of attention (Rosnick, Small, Graves & Mortimer, 2004) and individuals who report more pain perform more poorly on cognitive tasks than individuals who report less or no pain (Brown, Glass & Park, 2002; Sjogren, Olsen, Thomsen & Dalberg, 2000), the RAND general health and pain scores were used to control for any effects of perceived health and/or pain on cognitive performance.

Prospective and Retrospective Memory Questionnaire (PRMQ; Crawford, Smith, Maylor, Della Sala, & Logie, 2003) – this is a self-report measure of prospective and retrospective memory slips in everyday life. It consists of 16 items, 8 of which ask about prospective memory failures and 8 of which ask about retrospective memory failures. Participants are asked to indicate how often each memory mistake happens to them on a 5-point scale ranging from 5 (Very Often) to 1

(Never). Possible scores range from 8 to 40 for the retrospective and prospective memory scales. Higher scores indicate more frequent occurrences of memory slips. It has been suggested that researchers utilize multiple indicators of the constructs they are assessing to provide convergent validity (Crawford et al, 2003). Some research has reported a significant correlation between performance on formal measures of prospective and retrospective memory and subjective prospective and retrospective memory complaints, as assessed with the PRMQ (Kliegel & Jager, 2006), while other studies have not found such a relationship (Zeintl et al, 2006). The PRMQ was included in the present study as a means of exploring whether participants' subjective memory complaints were related to their performances on formal measures of prospective and retrospective memory.

Index of Estrogen Exposure (IEE; Smith et al., 1999) - an estimate of cumulative estrogen exposure was calculated for each participant based on Smith et al.'s (1999) IEE. As previously discussed, the markers of estrogen exposure incorporated into Smith et al.'s (1999) index have all been found to be clearly associated with estrogen levels. The markers included from Smith et al (1999) were: length of time on estrogen replacement therapy, age at menarche and menopause, parity, postmenopausal weight and time since menopause. For the purposes of the present study, postmenopausal body weight was recorded as body mass index (BMI). BMI takes into account differences in height and, as previously discussed, is significantly positively correlated with levels of

endogenous estrogen following menopause (Hankinson et al., 1995; Kaye, Folsom, Soler, Prineas, & Potter, 1991; Olson et al, 2006; Rannevik et a l., 1995). In addition, duration of breastfeeding was added to the IEE because, as was previously discussed, it has been shown to reduce cumulative exposure to endogenous estrogen (Bernstein, 2002).

The IEE was calculated by converting scores on the marker variables into standardized scores and adding or subtracting the scores as appropriate. Length of time on estrogen replacement therapy, age at menopause, and BMI were added to the index while age at menarche, parity, duration of breastfeeding and length of time since menopause were subtracted from the index.

Procedure

Women who were considered to be eligible for study inclusion during the initial telephone interview were invited to participate in the study. With the exception of two women who preferred to be tested at the University campus, the majority of participants completed the testing component of the study in their own homes. Written consent was obtained from all participants prior to testing. All measures were administered in a single test session of approximately an hour and a half. The order of interview and task administration was identical for all participants. A brief interview was conducted at the beginning of the test session in order to obtain demographic information, details of hormone and medication use and information regarding reproductive history (see Appendix B). Measurements of height and weight were also taken at this time. Upon

completion of the testing, participants were given feedback as to their performance and ten dollars to thank them for their participation.

CHAPTER 4

RESULTS

Timing of Estrogen Replacement

As previously discussed, it is possible that there is a "critical window" of time around the menopause when estrogen replacement may produce a protective effect on cognitive function in postmenopausal women (Brinton, 2004; Maki, 2006; Pinkerton & Henderson, 2005; Sherwin, 2006; Sturdee & Maclennan, 2006). In keeping with the "critical window hypothesis", it was decided to run all the statistical analyses twice; first, on the entire sample and secondly, on a reduced sample that excluded those women who would be considered "late initiators" of hormone therapy. For the purposes of the present study, "late initiators" were defined as women who had commenced estrogen replacement 5 or more years after menopause. The cutoff of 5 years was chosen based on MacLennan et al's (2006) criteria and Clarkson and Appt's (2005) review of estrogen and atherosclerosis in which they concluded that the beneficial effects of estrogen replacement against the development of coronary artery atherosclerosis are completely lost when treatment is delayed for six or more years after menopause.

Eliminating women who had taken estrogen replacement five or more years after menopause resulted in a reduced sample size of 50. One-way ANOVAS demonstrated that there were no significant differences between the group of 12 women who were removed from the full sample and the 50 women

in the reduced sample with regard to age, education, IQ, scores on the RAND General Health and Pain scales or scores on the GDS.

Means and standard deviations for the participant characteristics for this reduced sample are shown in Table 2.

Table 2: Participant characteristics of the reduced sample (N=50)

	Mean	Standard Deviation
Age	69.3	3.25
Education (yrs.)	15.1	2.7
IQ	102.8	16.1
GDS	1.6	2.0
RAND Health	77.6	14.4
RAND Pain	82.4	17.1

Note: IQ = intelligence quotient; GDS = score on the Geriatric Depression Scale; RAND Health = score on the Health scale of the RAND 36-Item Health Survey 1.0; RAND Pain = score on the Pain scale of the RAND 36-Item Health Survey 1.0. The General Health and Pain scales from the RAND are scored so that high scores indicate better health and less pain.

Differences between the full and reduced samples were examined with separate one-way analyses of variance (ANOVA) for age, education, IQ, RAND General Health, RAND Pain scores and scores on the GDS. No significant differences were found between the two groups on any of these variables.

Reproductive Characteristics of the Samples

Table 3 shows the means and standard deviations of the reproductive variables for the full and reduced samples respectively. Differences between the two samples were examined with separate one-way analyses of variance (ANOVA) for BMI, age at menarche, age at menopause, years since menopause, number of pregnancies, number of months spent breastfeeding and number of months of HRT use. No significant differences were found between the two groups on any of these variables.

Table 3: Means and standard deviations of scores of the reproductive variables for the full (N=62) and reduced samples (N=50)

	Full Sample		Reduced Sample	
	Mean	S.D.	Mean	S.D.
BMI	26.77	5.79	26.95	6.08
Age Mena	13.28	1.90	13.12	1.94
Age Meno	49.39	4.69	49.92	4.61
Time since mp	20.07	6.45	19.58	6.81
# Pregnancies	2.47	1.75	2.32	1.68
Time Bfeed	5.80	11.76	5.11	11.17
Time HRT	62.76	86.90	62.72	93.51

Note: BMI = body mass index; Age Mena = age at menarche in years; Age Meno = age at menopause in years; Time since mp = time since menopause in years; Time Bfeed = duration of breastfeeding in months; Time HRT = duration of hormone replacement therapy in months

Performance on Cognitive Measures

Table 4 shows the means and standard deviations for the cognitive measures for the full and reduced samples. Differences between the two samples were examined with separate one-way analyses of variance (ANOVA) for score on Logical Memory I and II, Digit Span and the prospective memory task. No significant differences were found between the two groups on any of these variables.

Table 4: Means and standard deviations of scores on the cognitive measures for the full (N=62) and reduced samples (N=50)

	Full Sample		Reduced Sample	
	Mean	S.D.	Mean	S.D.
LMI	42.47	9.13	42.60	9.80
LMII	25.82	7.61	26.12	7.78
Digit Span	6.65	1.22	6.70	1.23
ProMemory	5.02	1.41	5.0	1.44

Note: LM I = Logical Memory I score; LM II = Logical Memory II score; ProMemory = score on prospective memory task

Bivariate Correlations Between the Participant Characteristics and the Cognitive Measures

Prior to the main analyses, bivariate correlations were calculated between the cognitive measures and IQ, education, age, RAND General Health scores and RAND Pain scores and the IEE for each sample. This was done to avoid the

possibility that scores on these measures could confound any association between scores on the IEE and scores on the cognitive measure. Scores on the GDS were not included in this analysis as scores for all women in the study were well within the normal, nondepressed range with a score of 8 being the highest score obtained by the women in the sample. The bivariate correlations between the demographic and cognitive variables for the full sample are shown in Table 5 and the results for the reduced sample are shown in Table 6.

Table 5: Bivariate correlations for the participant characteristic variables, IEE and the cognitive measures for the full sample (N=62)

	Age	Educ	IQ	RGH	RP	DiSp	LMI	LMII	ProM
Educ	.045								
IQ	.074	.362[b]							
RGH	-.203	-.009	.099						
RP	-.050	.049	-.050	.440[b]					
DiSp	.240	.152	.095	-.160	.011				
LM I	-.141	.431[b]	.410[b]	-.052	.037	.074			
LM II	-.214	.386[b]	.347[b]	.114	.191	-.065	.814[b]		
ProM	.089	.039	-.067	-.057	-.095	.042	-.052	-.058	
IEE	-.356[b]	-.054	-.014	.029	-.006	.003	.006	.144	.186

Note: [b] = p<.01; Educ = education; IQ = intelligence quotient; RGH = RAND General Health subscale; RP = RAND Pain subscale; LMI = logical memory I; LMII = logical memory II; ProM = prospective memory task; IEE = index of cumulative estrogen exposure

Table 6: Bivariate correlations for the participant characteristic variables, IEE and the cognitive measures for the reduced sample (N=50)

	Age	Educ	IQ	RGH	RP	DiSp	LMI	LMII	ProM
Educ	-.017								
IQ	.031	.265							
RGH	-.220	-.031	.108						
RP	-.104	.122	-.034	.415[b]					
DiSp	.202	.162	.100	-.203	-.047				
LM I	-.187	.385[b]	.385[b]	-.073	.069	.076			
LM II	-.274	.376[b]	.337[a]	.117	.270	-.039	.835[b]		
ProM	.003	-.094	-.158	-.113	-.178	-.034	-.133	-.171	
IEE	-.471[b]	.006	-.047	.140	.125	.017	-.004	.158	.281[a]

Note: [a] = p<.05; b = p<.01; Educ = education; IQ = intelligence quotient; RGH = RAND General Health subscale; RP = RAND Pain subscale; LMI = logical memory I; LMII = logical memory II; ProM = prospective memory task; IEE = index of cumulative estrogen exposure

For the full sample education was significantly correlated with IQ (r = .362, p<.01), scores on the Logical Memory I subtest (r = .431, p<.01) and scores on the Logical Memory II subtest (r = .386, p<.01). IQ was significantly correlated with scores on the Logical Memory I subtest (r = .410, p<.01) and the Logical Memory II subtest (r = .347, p<.01). Scores on Logical Memory I were significantly correlated with scores on Logical Memory II (r = .814, r<.01). Scores

on the General Health subscale of the RAND were significantly correlated with scores on the Pain subscale of the RAND ($r = .440$, $p<.01$). Age was significantly correlated with scores on the IEE ($r = -.471$, $p<.01$). This was to be expected, given that time since menopause, which is one of the variables included in the IEE, would be influenced by age at the time of testing.

In the reduced sample, education was significantly correlated with scores on the Logical Memory I subtest ($r = .385$, $p<.01$) and the Logical Memory II subtest ($r = .376$, $p<.01$). IQ was correlated with scores on the Logical Memory I subtest ($r = .385$, $p<.01$) and the Logical Memory II subtest ($r = .337$, $p<.05$). Scores on the Logical Memory I subtest were correlated with scores on the Logical Memory II subtest ($r = .835$, $p<.01$). Scores on the General Health subscale of the RAND were significantly correlated with scores on the Pain subscale of the RAND ($r = .415$, $p<.01$). Age, as expected, was significantly correlated with the IEE. Scores on the Prospective Memory task were significantly correlated with the IEE ($r = .281$, $p<.05$).

Multivariate Multiple Regression Analyses

The relationships among the IEE, the control variables of education and IQ and the four dependent variables, Logical Memory I, Logical Memory II, Digit Span and the Prospective Memory task, were tested simultaneously using multivariate general linear analysis in SPSS. The memory scores were entered into the analyses as dependent variables while scores on the IEE, IQ and

education were entered as covariates. The results of the analyses are shown in tables 7 and 8 for the full and reduced samples respectively.

In both the full and reduced samples, education and IQ were not significant at the multivariate level in the analysis. IEE was not significant at the multivariate level in the full sample but was significant at the multivariate level in the reduced sample.

Table 7: Multivariate analysis of IEE, education and IQ as predictors of scores on Logical Memory I and II, Digit Span and prospective memory in the full sample (N=62)

Multivariate	Df	F	Wilks' λ	Hotelling's T	Sig.
IQ	4	1.47	.90	.11	>.05
Education	4	2.12	.87	.15	>.05
IEE	4	1.43	.91	.10	>.05

Table 8: Multivariate analysis of IEE, education and IQ as predictors of scores on Logical Memory I and II, Digit Span and prospective memory in the reduced sample (N=50)

Multivariate	Df	F	Wilks' λ	Hotelling's T	Sig.
IQ	4	1.38	.89	.13	>.05
Education	4	1.67	.87	.15	>.05
IEE	4	2.59	.81	.24	<.05

Results of the univariate analyses were consistent with the bivariate correlations. At the univariate level, IQ was significantly associated with Logical Memory I in the full ($F(1) = 5.80$, $p<.05$) and reduced sample ($F(1) = 5.18$, $p<.05$). In the full sample, education was significantly associated with Logical Memory I ($F(1) = 7.20$, $p<.01$) and Logical Memory II ($F(1) = 5.41$, $p<.05$). Education was also significantly associated with Logical Memory I ($F(1) = 5.17$, $p<.05$) and Logical Memory II ($F(1) = 5.16$, $p<.05$) in the reduced sample. IEE was significantly associated with Prospective Memory ($F(1) = 7.74$, $p<.05$) in the reduced but not the full sample.

Individual Markers of Estrogen Exposure and Prospective Memory

Given the significant association between the IEE and the Prospective Memory task in the reduced sample, bivariate correlations were calculated to determine whether any of the individual markers of estrogen exposure that comprised the IEE were related to performance on the Prospective Memory task. Bivariate correlations were calculated between length of time on estrogen

replacement therapy, age at menopause, BMI, age at menarche, parity, time since menopause, duration of breastfeeding and scores on the Prospective Memory task. A significant negative correlation was found between duration of breastfeeding and scores on the Prospective Memory task ($r = -.599$, $p<.01$), indicating that women with longer durations of breastfeeding had lower scores on the Prospective Memory task. BMI was significantly positively correlated with scores on the Prospective Memory task ($r = .275$, $p<.05$), such that women with higher BMIs obtained higher scores on the Prospective Memory task than women with lower BMIs.

Cumulative Endogenous Estrogen Exposure and Performance on Cognitive Measures

Previous studies have indicated that a higher level of cumulative exposure to endogenous estrogen is associated with better performance on cognitive measures later in life (Yaffe et al, 1999; Tan et al, 2004; Zhang et al, 2001). In keeping with this finding, all analyses were re-run on a third sample that only included women who had never taken any form of hormone replacement. Means and standard deviations for the participant characteristic variables for this endogenous estrogen only sample are shown in Table 9. One-way ANOVAS comparing the participant characteristic variables of the group of women who used estrogen (N= 34) and the group of women who had never used (N=28) estrogen showed that women who reported using estrogen also reported being healthier than women had never used estrogen ($F (61) = 6.16$, $p<.05$). No significant differences were observed between the estrogen users and nonusers

on the other variables.

Table 9: Participant characteristics of the endogenous estrogen only sample (N=28)

	Mean	Standard Deviation
Age	69.9	3.03
Education (yrs.)	15.1	2.8
IQ	105.1	17.0
GDS	1.5	1.8
RAND Health	73.8	15.6
RAND Pain	83.3	16.5

Note: IQ = intelligence quotient; GDS = score on the Geriatric Depression Scale; RAND Health = score on the Health sub scale; RAND Pain = score on the Pain subscale

The bivariate correlations between the participant characteristics, IEE and the cognitive variables for the endogenous estrogen only sample are shown in Table 10. In the endogenous estrogen only sample, education was significantly correlated with scores on the Logical Memory I subtest ($r = .385$, $p<.01$) and the Logical Memory II subtest ($r = .376$, $p<.01$). IQ was correlated with scores on the Logical Memory I subtest ($r = .385$, $p<.01$) and the Logical Memory II subtest ($r = .337$, $p<.05$). Scores on the Logical Memory I subtest were correlated with scores on the Logical Memory II subtest ($r = .835$, $p<.01$). Scores on the General Health subscale of the RAND were significantly correlated with scores on the Pain subscale of the RAND ($r = .413$, $p<.05$).

Table 10: Bivariate correlations for the participant characteristic variables, IEE and the cognitive measures for the endogenous estrogen only sample (N=28)

	Age	Educ	IQ	RANDG	RANDP	DigSpan	LM I	LM II
Educ	-.046							
IQ	-.117	.341						
RANDG	-.258	.006	.164					
RANDP	-.284	.211	-.079	.413*				
DigSpan	.256	.303	.280	-.234	-.130			
LM I	-.283	.522**	.393*	-.117	-.006	.227		
LM II	-.427*	.531**	.404*	.137	.350	-.002	.770**	
ProMem	.255	.039	-.114	-.264	-.343	-.091	-.210	-.287

Note: * = p<.05; ** = p<.01; Educ = education; IQ = intelligence quotient; RANDG = RAND General Health subscale; RANDP = RAND Pain subscale; LM I = logical memory I; LM II = logical memory II; ProMem = prospective memory task

The relationships among the IEE, the control variables of age, education and IQ and the four dependent variables, Logical Memory I, Logical Memory II, Digit Span and the Prospective Memory task were tested simultaneously using multivariate general linear analysis in SPSS. The result of the multivariate analysis is shown in table 11 for the endogenous estrogen only sample.

Table 11: Multivariate analysis of IEE, education and IQ as predictors of scores on Logical Memory I and II, Digit Span and prospective memory in the endogenous estrogen only sample (N=28)

Multivariate	Df	F	Wilks' λ	Hotelling's T	Sig.
Age	4	1.95	.72	.40	>.05
IQ	4	.99	.84	.20	>.05
Education	4	2.47	.70	.50	>.05
IEE	4	2.32	.68	.46	>.05

In the endogenous estrogen only sample, age, education, IQ and IEE were not significant at the multivariate level in the analysis. At the univariate level, age was significantly associated with scores on Logical Memory II ($F(1) = 6.25$, $p<.05$). In addition, education was significantly associated with scores on Logical Memory I ($F(1) = 7.30$, $p<.01$) and Logical Memory II ($F(1) = 7.46$, $p<.05$). IEE was significantly associated with scores on the Prospective Memory Task ($F(1) = 4.70$ $p<.05$).

Bivariate correlations were calculated between age at menopause, BMI, time spent breastfeeding, age at menarche, parity, time since menopause and scores on the Prospective memory task. BMI was significantly positively correlated with scores on the Prospective Memory task ($r = .465$, $p<.05$) while duration of breastfeeding was significantly negatively correlated with scores on

the Prospective Memory task ($r = -.644$, $p<.01$).

Self-reported Memory Complaints and Performance on Formal Measures of Retrospective and Prospective Memory

The means and standard deviations for scores on the retrospective and prospective memory scales of the PRMQ for the full sample are shown in Table 12. On average, participants reported experiencing prospective memory difficulties more frequently than retrospective memory difficulties ($t(61) = 5.66$, $p<.001$).

Table 12: Means and standard deviations of scores on the retrospective and prospective scales of the PRMQ for the full sample (N=62)

PRMQ Scale	Mean	Standard Deviation
Retrospective Memory	17.44	5.05
Prospective Memory	20.06	5.21

To see if any relationship existed between subjective memory complaints reported by participants on the PRMQ and their performance on formal measures of memory, bivariate correlations were calculated between scores on the retrospective memory scale of the PRMQ and scores on Logical Memory I and II and between scores on the prospective memory scale of the PRMQ and scores on the time-based prospective memory task. The bivariate correlations

between scores on the retrospective and prospective scales of the PRMQ and the formal memory measures are shown in Table 13.

No significant correlations were found between scores on the retrospective or prospective scales of the PRMQ and performance on any of the formal memory measures.

Table 13: Bivariate correlations for the retrospective and prospective scales of the PRMQ and the cognitive measures (N=62)

	PRMQ Retrospective	PRMQ Prospective
Logical Memory I	.035	.153
Logical Memory II	.020	.203
Digit Span	-.071	-.035
ProMem Task	.209	.209

Note: ProMem task = prospective memory task

No significant correlations were observed between scores on the retrospective or prospective scales or the total score from the PRMQ and scores on the IEE.

CHAPTER FIVE

DISCUSSION

The current life expectancy for women in Canada is 82.1 years (St-Arnaud, Beaudet & Tully, 2005). With an average age at menopause of 51 (Hogervorst et al, 2000), this means that most women will spend at least one-third of their lives in a postmenopausal hypoestrogenic condition. Although research in the basic sciences has established the biologically plausibility of estrogen having a beneficial effect on the brain structures and functions relevant for cognitive function, the research findings of the benefits of ERT in postmenopausal women are far from consistent. A number of explanations have been put forward to account for these inconsistencies. Of these, failure to consider the age at which a woman begins ERT, and failure to take into account the influence of variables known to affect endogenous estrogen exposure across the lifespan may be particularly relevant. The present study attempted to take both of these factors into consideration when examining the potential associations between cumulative estrogen exposure and prospective memory in older postmenopausal women. Prospective memory was chosen as a variable of interest due to its relevance for independent everyday function and its association with the PFC, an area of the brain on which estrogen is known to have effects.

Cumulative Estrogen Exposure and Prospective Memory

The primary purpose of this study was to determine if a relationship existed between the level of cumulative estrogen exposure a woman experiences

over her life and her performance in later life on a measure of prospective memory. Cumulative estrogen exposure was estimated by a mathematical index, the IEE, which included variables known to influence estrogen levels across the female lifespan, including age at menarche, age at menopause, parity, time since menopause, duration of breastfeeding, duration of ERT use and BMI. The results of the study suggested that cumulative estrogen exposure, as determined by the IEE, was associated with performance on the prospective memory measure, but only when women who had initiated ERT five or more years after the start of menopause were excluded from the analysis. Given that the subset of women who were removed from the data analysis did not differ from the principle sample with regard to variables known to influence performance on memory measures (for example, age, education and IQ), it would seem reasonable to suggest that the later timing of estrogen initiation in these women may account for the failure to find a positive relationship between the IEE and performance on the prospective memory task when they were included in the data analysis. It may also be the case that other differences between the main sample and the women who were excluded from the second set of analyses may have been responsible. However, the finding that excluding women who initiated ERT more than five years after menopause from the data analysis resulted in a significant positive correlation between cumulative estrogen exposure and performance on the prospective memory tasks is consistent with previous research that suggests that ERT is beneficial to cognitive functioning when

started shortly after menopause but may have no effect or be detrimental when commenced a number of years after menopause (Dunkin et al, 2005; MacLennan et al, 2006; Maki, 2005). Thus, the present results can be seen as supporting the concept of a "critical window" for the initiation of ER following menopause (Brinton, 2004; Maki, 2006; Pinkerton & Henderson, 2005; Sherwin, 2006; Sturdee & Maclennan, 2006). Based on previous research, it appears to be important to initiate estrogen early in menopause possibly before neurons lose their sensitivity to the effects of estrogen (Sherwin, 2005) and before disease processes have started that estrogen may be ineffectual in reversing or halting (Brinton, 2005).

Although the pattern of results found in the present study suggests that an earlier initiation of ERT following menopause may be beneficial for cognitive functioning later in life, unfortunately, the small sample size of the present study meant it was not statistically possible to compare the cognitive performance of the group of women who took estrogen within five years of menopause with that of the group of women who took ER five or more years after menopause. Such a comparison would have provided further information regarding the effect of the timing of ER initiation.

Cumulative Endogenous Estrogen Exposure and Prospective Memory

When the multivariate analysis was limited to the sample of women from the study that reported never using ER, the significant positive association between the IEE and performance of the prospective memory task that was

observed in the sample of women that included ER users, remained significant. This suggests that cumulative levels of endogenous estrogen are relevant for cognitive function in later life, a finding that is consistent with previous studies which have reported a positive association between cumulative endogenous estrogen and cognition (Tan et al, 2005; Yaffe et al, 1999; Zhang et al, 2001).

Although higher cumulative levels of endogenous estrogen appear to be beneficial for cognitive functioning later in life, it is unclear from the present study whether using ERT provides any additional benefit to women who already have a high cumulative level of endogenous estrogen exposure. However, Rasgon et al (2005) reported that women in their study with the longest duration of estrogen exposure, which was defined as length of reproductive period plus duration of postmenopausal ERT, also had the least cognitive impairment. This suggests that the use of ERT after menopause may extend the positive effects of endogenous estrogen. It has also been suggested that the clinical response of postmenopausal women to ERT may be influenced by reproductive and biological factors known to influence endogenous estrogen levels (Dunkin et al, 2005), which corresponds with Yaffe et al's (2007) suggestion that ER may be most effective in women with low endogenous levels of estrogen.

Individual Markers of Estrogen Exposure and Prospective Memory

In the present study, a significant negative association was observed between duration of breastfeeding and performance on the prospective memory measure, such that women who breastfed for longer durations of time were

found to perform less well on the prospective memory measure than women who did not breastfeed or breastfed for shorter durations. To the best of our knowledge, this is the first study to report an association between breastfeeding and cognition. While this finding should be considered preliminary and requires duplication, it is consistent with the studies that report a reduced risk of breast cancer in women who breastfeed for longer durations (Ma et al, 2006). It is thought that this reduction in risk is due to the reduced exposure to endogenous estrogen that accompanies breastfeeding (Bernstein, 2002).

BMI was significantly positively correlated with performance on the prospective memory task so that women with higher BMIs had higher scores on the prospective memory task. As BMI is considered a strong marker of endogenous estrogen in post-menopausal women (Grodstein et al, 2003), this suggests that women with higher postmenopausal BMIs have higher levels of endogenous estrogen than women with lower BMIs and that these higher levels of endogenous estrogen may have a positive effect on cognition. While the present finding is consistent with previous studies that have found a positive relationship between BMI and cognitive performance in postmenopausal women (Patel et al, 2004), it is important to acknowledge that a number of studies have reported a significant positive correlation between an individual's BMI and the risk of developing AD (Gorospe & Dave, 2007). It is likely that there is a point at which the potential benefits of the higher levels of endogenous estrogen that come from being overweight and obese, are outweighed by the negative health

effects of carrying excess weight. There is research to support this idea. In their study of elderly French men and women, Deschamps et al (2002) found that individuals with a BMI between 23 and 27 kg/m^2 had the lowest risk of cognitive and functional decline compared to individuals with higher or lower BMIs. A similar U-shaped relationship has been observed between BMI and the risk of becoming dependent for activities of daily living in older women such that underweight and obese women were more likely to become dependent than women in the normal BMI range (Wilkins & de Groh, 2005). On average, women in the present study had a BMI of 26.8 kg/m^2 (26.9 kg/m^2 for the reduced sample). Based on the findings of Dechamps et al (2002), this would suggest that women in the present study were, on average, at an optimal BMI for reducing their risk of cognitive decline. This may account for the positive correlation observed between BMI and performance on the prospective memory task in the present study. Unfortunately, the present sample size was insufficient to determine whether the U-shaped relationship between BMI and cognition reported by Dechamps et al (2002) existed between BMI and prospective memory performance.

It is also important to acknowledge that although the present results suggest a relationship between postmenopausal BMI and prospective memory, it is also possible that there may be other variables that are related to BMI which could influence cognitive function that were not statistically controlled for in the present study.

Cumulative Estrogen Exposure and Verbal Memory

The finding of no relationship between cumulative estrogen exposure and verbal memory in any of the samples analyzed in this study was unexpected given the numerous observational ERT studies that report a positive effect of estrogen on verbal memory (Ghidoni et al, 2006; Jacobs et al., 1998; Kampen & Sherwin, 1994; Maki, Zonderman & Resnick, 2001; Resnick, Maki, Golski, Kraut, & Zonderman, 1998: Shaywitz et al, 2003; Stephens, Hamilton & Pachana, 2003). There are a number of possible reasons why such an effect was not observed. For example, it has been theorized that deficits in frontal lobe mediated cognitive functions may occur earlier in the course of aging than deficits in cognitive abilities that are believed to rely on the hippocampus (West, 2000). In support of this theory, Elsabagh, Hartley and File (2007) recently reported that women in the later stages of menopause exhibited deficits on measures of executive function (planning and mental flexibility) but not memory relative to women in earlier stages of menopause. These differences were independent of age, leaving Elsabagh et al (2007) to conclude that the pattern of results obtained by the women in their study suggest that executive functions deteriorate more quickly than other cognitive functions and that the differences in executive functioning observed between early and late menopause may be due to the differences in levels of estrogen that exist between these two stages of menopause. As executive functions such as planning and mental flexibility are thought to rely on the frontal lobes (Royall, Palmer, Chiodo & Polk, 2004), Elsabagh et al's (2007)

findings are evidence that deficits in frontal-mediated cognitive functions, such a prospective memory, may be observed earlier in the aging process than deficits in hippocampal-mediated functions, such as verbal memory.

A number of other studies have reported finding a positive effect of ERT on cognitive functions related to the frontal cortex while failing to find similar effects of ERT on tasks that rely on the hippocampus (Duff & Hampson, 2000; Joffe et al, 2006; Keenan et al, 2001; Krug et al, 2006). Keenan et al (2001) have suggested that the frontal cortex is the primary site for estrogen's effects on cognition and the results of the present study are consistent with this theory.

Subjective Memory Complaints and Objective Measures of Memory

Consistent with previous findings using the PRMQ, women in the study reported more problems with prospective than retrospective memory (Kliegel & Jager, 2006). However, no association between subjective memory complaints, as assessed with the PRMQ, and performance on the standardized and laboratory memory tasks included in this study were observed. The failure to find a correlation between subjective complaints of retrospective memory and performance on objective measures of memory in older women is consistent with the findings from previous research (Pearman & Storandt, 2004). It appears that in middle-aged and young elderly individuals, subjective memory complaints are more likely to reflect depression, anxiety or personality factors than actual memory impairment (Jonker, Geerlings & Schmand, 2000). In addition, female

gender has also been associated with a high prevalence of memory complaints (Jonker et al, 2000).

The majority of previous studies have focused on self-reported retrospective memory problems and few studies have looked specifically at the relationship between self-reported prospective memory difficulties and performance on objective measures of prospective memory (Zeintl et al, 2006). Two studies have reported a positive association between scores on the prospective scale of the PRMQ and performance on objective measures of prospective memory (Kliegel & Jager, 2006; Mantyla, 2003). A third study by Zeintl et al (2006) found no correlation between scores on the prospective memory scale of the PRMQ and objective prospective memory performance. Instead, Zeintl et al (2006) found a significant positive relationship between self-reported prospective memory complaints and scores on the GDS, suggesting that, as was the case with retrospective memory complaints, prospective memory complaints are more reflective of psychological factors than objective memory impairment. While none of the women in the present study acknowledged being depressed or had scores on the GDS that were suggestive of even mild depression, a significant positive correlation was observed between the scores these women obtained on the GDS and scores on the retrospective ($r = .286$, $p<.05$) and prospective ($r = .413$, $p<.01$) memory scales of the GDS. The correlations between scores on the retrospective and prospective memory scales of the PRMQ and performance on the objective memory measures remained

nonsignificant after statistically controlling for any possible confounding effects associated with scores on the GDS. This pattern of results suggests that differences in scores on the GDS were not responsible for the failure to find a relationship between self-ratings of prospective memory and objective prospective memory performance.

An alternative explanation for our failure to find a correlation between self-reported prospective memory complaints and actual prospective memory performance may be that, as Zeintl et al (2006) suggest, experimental measures of prospective memory, such as the one used in the present study, do not reflect the types of everyday memory failures assessed in the PRMQ. The finding that scores on the IEE were significantly associated with scores on the prospective memory task but not with scores on the prospective memory scale of the PRMQ also suggests that the PRMQ and the prospective memory task may be tapping different constructs. Future studies that utilize naturalistic rather than laboratory-based memory tasks would be beneficial in clarifying the relationship between the PRMQ and objective memory performance.

Limitations of the Present Study

When interpreting the results of the present study, it is important to be aware of some of the inherent limitations of the study. Firstly, this study was retrospective in nature and thus relied on women's recall of information such as age at menarche and menopause. In addition, the lack of experimental design makes it difficult to determine whether differences in cognitive performance are

solely attributable to differences in cumulative estrogen exposure or are the result of other variables that were not measured or accounted for in the statistical analyses. In addition, women's recall of ERT was not validated against pharmacy or prescription records. However, it is worth noting that MacLennan et al (2006) reported that 94% of their participants' responses matched medical records for hormone therapy type, route and dose. They also found a high correlation between participants and their doctors regarding duration of hormone use. These findings suggest that, in the present healthy nondemented group of women, recall of hormone use may have been fairly accurate.

From a statistical perspective, the small sample size used in this study was also a limiting factor in that the study was underpowered to examine the effects of other variables that may have influenced the findings of the study. For example, it was not possible to distinguish the effects of unopposed estrogen from those of estrogen and progestin. As previously discussed, MPA, one of the progestins most commonly used in combination with estrogen, has been shown *in vitro* to mitigate the protective effects of estrogen (Nilsen & Brinton, 2002). In addition, given that the majority of women in the present study reported using an oral form of ERT, it was not possible to examine the effects of route of ERT administration on cognitive function. This may have been relevant given that previous research suggests that transdermal administration of estrogen may have more beneficial effects on cognitive function that oral administration (Gleason et al, 2004).

APOE ε4-carrier status was not determined in the present study. As previously discussed, ERT either has no effect (Burkhardt et al, 2004) or increases cognitive impairment (Yaffe et al, 2000) in women who test positive for the APOE ε4 allele. More importantly, Driscoll, McDaniel and Guynn (2005) recently observed a significant deficit in prospective memory performance for healthy elderly individuals in their study who were APOE ε4 carriers relative to those individuals who tested negative for the APOE ε4 allele.

The applicability of the present findings to other postmenopausal women is also questionable given that the women in the present study were generally in good health and well-educated (the mean number of years of education for the entire sample was 15) and as a result may not be truly representative of the population of postmenopausal women in general.

It is also unclear to what degree the prospective memory task utilized in the present study reflects everyday prospective memory. Looking at the effects of cumulative estrogen exposure on naturalistic tasks of prospective memory versus laboratory tasks would increase the ecological validity of the present findings.

Summary and Suggestions for Future Research

Although the findings of the present study are of considerable interest and correspond with the current research literature regarding ERT in postmenopausal women, they need to be replicated in a larger, perhaps more diverse sample of postmenopausal women. In addition, prospective studies that

follow women from perimenopause would help to reduce some of the potentially confounding variables that can occur in retrospective studies such as the present study. Such studies are now underway including the Kronos Early Estrogen Prevention Study (KEEPS) which is a 5-year, randomized clinical trail designed to assess the effects of transdermal estradiol and oral CEEs on cardiovascular disease in women who will be within 36 months of their final menstrual period at the initiation of treatment (Harman et al, 2005). While its primary goal is examining the effects of ERT on risk factors for cardiovascular disease, ancillary studies have been planned that will look at the effects of ERT on cognition.

The duration of the KEEPS study is five years, which means that the majority of the women in the study will be less than 60 years old by the end of the study. Additional studies that follow women who use ERT in the early menopausal years are needed to allow researchers to examine the effects of early ERT on cognitive functioning at later stages in life and to help clarify the issue of what aspects of cognition ERT may help to maintain during the aging process. In that regard, it will be important for future studies to include cognitive measures that are specifically sensitive to the hippocampus and to the frontal cortex.

The findings of this study, along with those of a number of other studies (Dunkin et al, 2005; Rasgon et al, 2005; Smith et al, 1999), indicate the importance of recognizing factors that influence levels of endogenous estrogen and how they may influence cognitive outcome in later life as well as modify the response to

ERT. As stated by the North American Menopause Society (2007) in their most recent position statement on the use of estrogen and progesterone in perimenopausal and postmenopausal women, additional studies are needed that specifically examine the effects of endogenous estrogen on clinical outcomes.

To summarize, the results of the present study suggest that the degree of cumulative lifetime exposure to estrogen a woman has may influence her prospective memory performance later in life. The pattern of results also suggests that the timing of ERT and the influence of reproductive and biological markers of endogenous estrogen exposure are relevant factors to consider when studying estrogen's effects on cognitive functioning in postmenopausal women. However, given the numerous inconsistencies that exist within the ERT literature, the present results must be viewed as preliminary and require replication.

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APPENDIX A: TELEPHONE INTERVIEW SHEET

Name: _____

Phone Number: _____

D.O.B.: _____

Hysterectomy? No _____ Yes _____ Bilateral oopherectomy? Yes ____ No ____

Do you consider yourself in good physical and mental health? Yes ____ No _____

Any history of physical or mental illness? Yes _____ No _____ (if yes, get details and dates) _____

Can you recall how old you were you when you reached menopause? Yes ____ No ____

Have you ever taken any form of hormone replacement? Yes _____ No _____

Will you be able to provide information regarding type, duration and timing of HR use? Yes _____ No _____

Current Medications:

_____

_____

_____

(After describing the study requirements and informing the person that full consent will be obtained at the time of testing)

Are you willing and able to take 1 and ½ to 2 hours to complete the study requirements? Yes _____ No _____

Are you willing for the researcher to take a measure of your height and weight? Yes _____ No _____

Would you prefer to be tested in your own home ____ or in the researcher's office ____ ?

Would you prefer mornings _____ afternoons _____ or evenings _____ ?

Are there particular days that you would not be available? Yes _____ No _____

(If yes) Which days _____

Are there particular days that you would prefer? Yes _____ No _____

(If yes) Which days _____

The person will then be told that they will be contacted within the next week to arrange a suitable time for a testing session.

APPENDIX B: IN-PERSON INTERVIEW PROTOCOL

Participant Number: _____

D.O.B. _____

Current medications (name and dosage, what for): _____

_____

_____

_____

Have you ever taken hormone replacement (HRT)? YES _____ NO _____

(If participant has taken HRT on several occasions, be sure to get dates, type of HRT and duration for each time)

When did you take hormone replacement?

_____

_____

_____

How long did you take hormone replacement?

_____

_____

_____

What type(s) of hormone replacement did you take?

_____

_____

Age at Menarche: _____

Age at Menopause: _____

Number of full-term pregnancies: _____

Number of children breast-fed _____

Total number of months of breast-feeding _____

Height: _____

Weight: _____

Have you generally been this weight since menopause? YES _____ NO _____ **(if NO, ask the person to estimate what their average weight has been since menopause:**

_____

Education: _____