The Role of Estrogens in Cognition: Does Prophylactic Oophorectomy Affect Verbal, Spatial and Working Memory?

by

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Abstract

Studies of non-human animals have shown that estrogens have a significant effect on the structure and neurochemistry of the brain and on cognitive behaviours. This study examines women with BRCA1/2 mutations who have undergone bilateral salpingo-oophorectomy (BSO), resulting in surgical menopause. In order to understand how the absence of endogenous estrogens affects cognition, women with BSO are compared to women with BRCA1/2 mutations but without BSO, as well as age-matched controls on tests of verbal, spatial and working memory. Women with BSO show decreased verbal memory and attention (logical memory) relative to controls but better performance on a measure of temporal lobe function (fluency clustering). Further, concentration of estrogen metabolites (E1G) negatively correlated with performance on a spatial memory task (object placement task) and with fluency clustering. These results indicate that endogenous estrogens are important for verbal memory and attention, but may contribute negatively to spatial memory and fluency clustering.
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Chapter 1: Introduction

Sex differences have been identified between men and women in a variety of different perceptual and cognitive modalities including speed and perceptual accuracy, fine motor skills, verbal abilities, spatial cognition and working memory (Sherwin, 1997; Hampson, 1995; Duff & Hampson, 2001). Menstrual cycle studies, which compare cognitive performance at different points in the menstrual cycle, have also found changes in performance of verbal and working memory tasks (Hampson, 1990; Hatta & Nagaya, 2009; Hollander et al., 2005). Taken together, these streams of research indicate that various forms of cognition may be influenced by differential activations of endogenous sex steroids (namely estrogens, androgens and progesterone) in the brain. Further, both animal and human studies have shown that steroid hormones act on the brain directly through receptors, found throughout the cortex, hippocampus and hypothalamus, as well as via second messenger systems, and through interactions with neurotransmitter systems (Bixo et al., 1995; MacLusky et al., 1986; MacLusky et al., 1987; McEwen & Alves, 1999; Sherwin, 2003, Woolley & McEwen, 1992).

In 1990, Gould, Woolley, Frankfurt & McEwen, introduced evidence that steroid hormones have a direct relationship to regions of the brain not generally implicated in reproductive functions. Their study of rats showed that the surgical removal of the ovaries, ovariectomy, resulted in a profound reduction in dendritic spine density in CA1 pyramidal neurons of the hippocampus. Estradiol injections prevented this decrease to such an extent that there was no difference in synaptic density between estradiol treated ovariectomized rats and intact rats. Effects of estradiol were enhanced when progesterone was added to the estradiol injection. Similar effects of estradiol were not found in CA3 pyramidal neurons or dentate gyrus granule cells, suggesting that the effects of steroid hormones are specific to CA1 pyramidal neurons. While previous studies had demonstrated a direct relationship between steroid hormones and cellular changes in the mammalian brain, this was the first study to demonstrate such a relationship in an area primarily associated with cognition.

While morphological fluctuations mediated by steroid hormones are profound (Desmond & Levy, 1997), it is the association between hormone levels and behavioural effects that are of particular interest when studying the human female brain. Rodent models indicate that the
actions of estrogens on hippocampal neurons result in a behavioural benefit in cognitive domains that have been localized to the hippocampus. Ovariectomized rats treated with estradiol injections show improved spatial skills (Frick et al., 2002; Packard & Teather, 1997) and spatial learning (McLaughlin et al., 2008).

Humans also show effects of estrogen replacement and deprivation, most notably in the area of verbal memory (Hampson, 2008; Sherwin, 2003). Studies on the effects of steroid hormones on cognition in humans generally use one of four designs: sex differences studies, menstrual cycle effects, effects of hormone replacement therapy (HRT) or changes following surgical menopause. In sex differences studies, male and female performance on cognitive tasks are compared. In the menstrual cycle paradigm, behavioural performance is measured at number of points in the menstrual cycle, most commonly during the midluteal phase, when estrogen is high, and during the follicular phase, when estrogen is low, and then compared. Cognitive abilities that fluctuate over the course of the menstrual cycle are believed to be influenced by ovarian hormones. In HRT paradigms, postmenopausal women taking HRT are compared to postmenopausal women not taking HRT on cognitive tasks. HRT studies are sometimes randomly controlled trials (RCTs), where women are randomly assigned to a condition, and sometimes observational studies, where women self-select to take HRT for personal reasons and are subsequently recruited for study participation. In the surgical menopause paradigm, the effects of hormones on cognition are studied in women without a primary source of endogenous estrogens. Women who have undergone surgical removal of their ovaries and fallopian tubes (bilateral salpingo-oophorectomies, BSO) experience a rapid and abrupt loss of ovarian steroids (namely 17β estradiol, testosterone and progesterone; Sluijmer et al., 1995), resulting in surgical menopause (Henderson & Sherwin, 2007). Evidence for the behavioural effects of estrogen on different cognitive functions in a variety of brain areas will be presented below.

1 Hippocampus

Rodent studies indicate that hippocampal neurons are sensitive to changes in endogenous and exogenous estrogens (Woolley & McEwen, 1992; Woolley 1998; Wong & Moss, 1992). In humans, estrogenic effects on memory are suggested by sex difference paradigms in which women score higher than men (Hampson, 2008; Sherwin, 1997), and menstrual cycle paradigms where verbal memory scores are greatest during ovulation when estrogen levels are high.
(Hampson, 1990). Additionally, brain activity in regions that subserve verbal memory, such as the hippocampus, parahippocampus, left inferior frontal gyrus, fluctuate with plasma estradiol levels (Craig et al., 2008; Maki & Resnick, 2000; Resnick et al., 1998).

Studies in postmenopausal women using HRT show a positive correlation between HRT use and hippocampal volume (Eberling et al., 2003) as well as improved scores on tests of verbal memory (Duka et al., 2000; Jacobs et al., 1998; Maki et al., 2001; Resnick et al., 1998; Sherwin, 2005; Tierney et al., 2009; Zec & Trivedi, 2002).

However, the literature in humans is controversial as other studies have shown a negative relationship between HRT use and cognitive scores (Espeland et al., 2004, File et al., 2002) or no relationship at all (Binder et al., 2001; Dumas et al., 2006; Joffe et al., 2006). Perhaps the most negative study was the Women’s Health Initiative Memory Study (WHIMS) a corollary to the Women’s Health Initiative (WHI), the largest RCT ever undertaken for HRT, concluded that HRT has an adverse effect on cognition in postmenopausal women (Espeland et al., 2004; Shumaker et al., 2003). These results, in combination with the main WHI studies, which concluded that HRT increased incidence of cardiovascular disease, stroke and breast cancer in menopausal women (Anderson et al., 2004; Rossouw et al., 2002), had a huge negative impact on the general population’s view of HRT.

This discrepancy in findings may speak to the methodological limitations of the HRT paradigm for assessing the influence of estrogens on cognition. To begin, HRT studies use a variety of different hormone preparations including: 17β estradiol (E2; Duka et al., 2000), conjugated equine estrogen (CEE; Espeland et al., 2004) and a combination of CEE and medroxyprogesterone acetate (MPA; Shumaker et al., 2003). Studies using E2 consistently show better results in regards to cognitive performance likely because E2 diffuses into the brain more easily than estrone (E1, the major metabolite in CEE; Steingold et al., 1986; Sherwin, 2005). This finding is supported by animal studies that show that fluctuations in the excitability of CA1 pyramidal neurons are specific to E2 (Wong & Moss, 1992).

A second limitation with HRT studies is that they use a variety of means of hormonal administration: oral (Binder et al., 2001), intramuscular (Phillips & Sherwin, 1992; Sherwin,
1988) and transdermal (Duka et al., 2000). Because HRT studies that use intramuscular and transdermal routes of hormone administration consistently find more significant results on cognitive measures, it may be that oral administration is not as effective as more direct routes (Sherwin, 2005).

A third limitation of HRT studies is the tests used to measure cognitive abilities. Cognition studies are often created as an adjunct to broader health studies and many of the neuropsychological tests used to assess cognition are not sensitive enough to the specific changes one can reasonably expect to see in the absence of estrogen. For example the Mini–Mental States examination (3MSE), a test of global functioning generally used to diagnose dementia, is often used to assess cognition in HRT studies (Espeland et al., 2004, Mc Lay et al., 2003). However, the 3MSE is not sensitive enough detect small cognitive changes, as scores do not fluctuate until a substantial amount of cortical degradation has occurred (Strauss et al., 2006).

Finally, many researchers have advanced the hypothesis that hormone replacement is most effective when it is administered immediately following estrogen deprivation (Marder & Sano, 2000; Sherwin, 2007). This hypothesis, known as the critical period hypothesis, is supported by animal studies that show that the effectiveness of estrogen replacement is substantially reduced as time post ovariectomy is increased (Daniel et al., 2006; Markowaska & Savonenko, 2002). In humans, the most consistent effects of HRT are found in surgically menopausal women, who are abruptly deprived of endogenous estrogens, and then immediately begin a replacement regime (Henderson & Sherwin, 2007; Vearncombe & Pachana, 2009). Most HRT studies, both those with significant results and those without, look at cohorts of women who have already been postmenopausal for a substantial period of time (Zec & Trivedi, 2002). Taken together, these limitations can account for much of the substantial variations of findings in the HRT literature.

The WHIMS studies are problematic for many of the reasons discussed above: the women in the studies were administered CEE and CEE+ MPA, rather than E2; the hormones were administered orally; only the 3MSE was used to measure cognitive functioning and the mean age of the women in both studies was 73, meaning the women had already been postmenopausal for some time before beginning HRT. These limitations question the appropriateness of
generalizing the findings of WHIMS to other populations. Taken together, these limitations can account for much the substantial variation in findings with the HRT literature.

The use of surgically menopausal women to study the effects of estrogens on cognition has addressed some of the methodological limitations presented by the HRT literature. It is an especially helpful paradigm because it avoids confounding the effects of E2-deprivation with the effects of aging. In 1988, Barbara Sherwin conducted the first study examining the relationship between estrogens and cognition in surgically menopausal women. Three months following BSO, women who were given one of three hormonal treatments (androgen-E2, E2-alone or androgen-alone) performed better than women who were given a placebo on the Wechsler Memory Scale (WMS) digit span and logical memory subtests, tests of working and verbal memory, respectively. Further studies replicated the positive effects of E2 replacement on verbal memory, but not working memory two months post-surgery (Phillips & Sherwin, 1992) as well as ten or more years post-surgery (Verghese et al., 2000). One limitation to these studies is that they show the effect of exogenous estrogens rather than endogenous estrogens. As spine and dendritic density fluctuate with the estrus cycle in rat models (Woolley & McEwen, 1992), naturally cycling estrogens may have different cognitive effects than steady-state hormonal treatments. An alternative study design compares surgically menopausal women to naturally menopausal controls. As the post menopausal ovary still releases hormones (Fogle et al., 2007; Laughlin et al., 2000), surgically menopausal women have lower hormone levels than women the same age who have intact ovaries, even if those women are already naturally postmenopausal. Observational studies using this design also find a positive relationship between estrogens and verbal memory such that naturally menopausal women have significantly better verbal memory scores (Farrag et al., 2002; Nappi et al., 1999).

The hippocampus is also important for different kinds of spatial cognition: encoding and retrieving episodic memories set in a temporal-spatial context (Rauchs et al., 2007), memory for object location (Crane and Milner, 2005) and path learning (O’Keefe, 1978). While men perform better than women on tests of spatial perception such as mental rotation (Hampson, 1995; Silverman et al., 2007), women perform better than men on tests of object location memory (Honda & Nihei, 2009; Spiers et al., 2008). Menstrual cycle studies indicate that spatial abilities such as mental rotation are improved when E2 levels are low (Silverman & Phillips, 1993;
Hampson, 2008). The corollary relationship between object location memory and E2 has never been studied.

2 Prefrontal Cortex

The working memory (WM) system involves the manipulation of a limited number of items while they are held in mind (Baddley, 2003; Goldman-Rakic, 1993). Positron emission tomography (PET) studies show that the mid dorsolateral prefrontal cortex (PFC) is implicated in working memory tasks, particularly the manipulation component (Petrides et al., 1993; Petrides, 1995). Aromatase complexes and estrogen receptors have been found in the prefrontal cortex of rats (McEwen & Alves, 1999) monkeys (MacLusky et al., 1986) and humans (Bixo et al., 1995). As well, estradiol treatments improve working memory performance in female rhesus monkeys and rodents (Biomonte & Denenberg, 1999; Daniel et al., 2006; Tinkler & Voyoto, 2005). In humans, estradiol concentrations in the PFC of women of reproductive age are significantly higher than in postmenopausal women (Bixo et al., 1995).

Duff & Hampson (2000) speculate that estrogen may contribute to WM specifically by influencing the active manipulation of items in mind. As such, tasks that involve simply holding items in memory, such as Digits Forward (from the Wechsler Memory Scale) should not be affected by estrogen levels, whereas tasks that require holding items in memory and manipulating them should. Duff and Hampson (2001) demonstrates a female advantage sex differences on a task of working memory with a manipulation component (Digit Ordering Task, Spatial Working Memory Task) but not on working memory tasks without (Digits Forward, Corsi Block Tapping Test). This hypothesis is supported by HRT studies, which show improved performance on working memory tasks with estrogen treatments (Duff & Hampson, 2000; Krug et al., 2006; Phillips & Sherwin, 1992).

3 Surgical Menopause Population: BRCA1 and BRCA2

One of the primary benefits to the surgical menopause paradigm is that is helps shed light on how naturally occurring hormones influence cognition in the female brain without the confounds of aging effects. As discussed above, HRT paradigms present challenges in how widely their results can be generalized. In order to better understand the role of naturally occurring endogenous estrogens in cognition in middle aged women, we used a paradigm that included 3
populations: women with a BRCA1/2 mutation who have undergone prophylactic oophorectomy (BSO); women with a BRCA1/2 mutation who have not undergone BSO and age-matched controls for the surgically menopausal women.

Women with Breast Cancer 1 and Breast Cancer 2 (BRCA1/2) mutations are at an elevated lifetime risk of developing breast and ovarian cancer. Lifetime risk of breast cancer is estimated at 45-87%, or ten times that of the general population, while lifetime risk of ovarian cancer is estimated at 15-45% (Antoniou, et al., 2003; Chen & Parmigiani, 2007; Ford, et al., 1998; Narod, 2006). BRCA1/2 are tumour suppressor genes, whose proteins carry out a number of tasks including DNA repair during homologous recombination (Narod, 2002). The BRCA1 protein is also involved in the regulation of estrogen receptor activity and is a co-activator of the androgen receptor (Narod, 2002). The mutations, discovered in 1994 and 1995, are not sex-linked, and thus can be passed on through the paternal side of the family as well (Kauff & Barakat, 2007; Metcalfe et al., 2010).

Three predictors of breast cancer risk in women are: age of menarche and age at which first child was born and age of menopause (Pike et al., 1983). The directionality of these risks - the younger the age of menarche, the older the age of first pregnancy, and the later the age of menopause, the greater the risk – suggest that lifetime hormone quantities, particularly of estrogens, play a role in the development of breast cancer. Women with BRCA1 mutations show a similar relationship between breast cancer risk and estrogen exposure. Lifetime risk of developing breast cancer peaks between the ages of 40 and 55, and decreases after menopause (Narod, 2006). Early menarche and use of the oral contraceptive pill both increase risk of breast cancer in BRCA1 carriers (Narod, 2002; Narod, 2006). As such there is a means to believe that the removal of the ovaries, by BSO may reduce risk of subsequent development of not just ovarian cancer but breast cancer as well.

Epidemiological studies confirm that BSO reduces breast cancer by 50% (Eisen et al, 2005; Rebbeck et al., 2002) and ovarian, fallopian and peritoneal cancers by 80% (Finch et al., 2006). For optimal protection, BSO is recommended before age 40, particularly for BRCA1 mutation carriers (Eisen et al., 2005; Kauff & Barakat, 2007; Kurian et al., 2010), meaning that women
will undergo surgical menopause as many as ten years before they would enter natural menopause.

4 Estrogen-Deprivation and Alzheimer’s Disease

While studies in the short-term have found decreased performance on verbal and working memory in the 6 months following oophorectomy (Sherwin, 1988; Phillips & Sherwin, 1992; Farrag et al., 2002), epidemiological studies have found that women who had oophorectomies prior to natural menopause are at elevated risk of developing dementia (Rocca et al., 2007). Further, the younger the women were at the time of surgery, the greater their increase in risk. Additionally, women who lost both ovaries to surgery had a greater risk of developing dementia than women who only lost one ovary. These results suggest that estrogens have neuroprotective effects in the female brain, a conclusion that is supported by a number of animal models (Resnick & Henderson, 2002).

The role of estrogen in women’s brain health may help explain why Alzheimer’s Disease (AD) is twice as prevalent in women as it is in men (Henderson et al., 2000). In fact, being female is considered a risk factor for developing AD (Raber, 2008). However, less is known about how knowledge of animal models of estrogen neuroprotection can be used to prevent or treat AD in humans. Use of estrogen replacement therapy (ERT) as a means of treating AD has had mixed results. Many studies find that estrogen therapy does nothing to ameliorate the effects of AD (Henderson et al., 2000; Mulnard et al., 2000; Wang et al., 2000). At least one study, which uses a higher dose of ERT (0.10mg/day of 17β estradiol) administered transdermally, has found positive effects of ERT on attention, verbal, visual and semantic memory (Asthana et al., 2001). This study suggests that some of the null results depicted in other studies of ERT as a means of AD treatment may be the result of specifics of the methodology, namely the type of estrogen used, dose size and means of administration. In addition, as iterated in the discussion of the critical time period hypothesis above, it may be that ERT is most effective when administered at the outset of perimenopause, making ERT more effective as a means of AD prevention rather than treatment. A case-control study in Rochester, Minnesota (Waring et al., 1999) comparing women with AD to age-matched women without, found that the frequency of ERT use was higher among the control subjects than AD patients. Further, the Cache County Study (Zadhi et al., 2002), which examines HRT rather than ERT use, suggests that long-term post-menopausal
HRT use of 10 years or more could reduce the sex-specific increase in risk for AD in women. While this study does not discriminate between hormone replacements with or without progesterone, 72% of the HRT group were taking unopposed oral estrogens.

A second explanation for the equivocal effects of HRT on prevention and slowing of AD is that HRT effectiveness is mediated by apolipoprotein E (APOE) genotype (MacLusky, 2004). The APOE gene has three allelic variations: E2, E3 and E4, which code for the proteins, e2, e3 and e4 respectively. The APOE genotype E4 is associated with an increase risk of AD. Corder et al. (1993) found that 91% of individuals carrying two E4 alleles developed AD by age 80. The incidence for individuals with one E4 allele is markedly lower, at 47%. E4 is also associated with the progression of multiple sclerosis (Fazekas et al., 2001) and Parkinson’s Disease (Tang et al., 2002)

One proposed mechanism by which APOE plays a role in cognitive decline, is via the effects of 17β estradiol (MacLusky, 2004; Struble et al., 2003). A series of studies in mice demonstrate that APOE protein expression is dependent on estradiol. APOE levels vary during the estrous cycle, and increase following administration of 17βestradiol (Struble et al., 2003). In APOE knock-out mice, addition of human e3 increased neurite growth while e4 decreased growth (Nathan et al., 2002). While 17β estradiol increased neurite growth in e2 and e3 mice, it did not in e4 mice (Nathan et al., 2004). Further, estradiol increased neurite growth more in e2 mice than in e3 mice, a genetic modulation that may explain why e2 has been associated with reduced risk of AD (Farlow, 1997; MacLusky, 2004). Taken together these studies suggest that the effects of estradiol on neuronal growth are mediated by an APOE-dependent mechanism. This theory is supported by research in human populations, which shows that ERT use reduces cognitive decline only in non-E4 women (Yaffe et al., 2000).

Combined, the animal and epidemiological literature strongly suggests that the interactions between APOE and estradiol have consequences for female brain health. A surgically menopausal population presents a unique opportunity to the study the relationship between genetic and hormonal factors on the development of AD. As decreased performance on hippocampal tasks is one of the earliest signs of cognitive change leading to AD (Bondi et al., 1999; Hyman et al., 1984; Tierney et al., 2005), studying cognitive change in a population of
women without a primary source of endogenous estrogens will help clarify the neuroprotective role of estrogens in the female brain while assessing the risk of dementia in this population. Further, comparisons of cognitive performance of women with E4 and non-E4 alleles will help clarify the behavioural consequences of an apoE-estradiol interaction model.

In order to (1) detect cognitive changes that occurred with rapid and sustained early loss of endogenous estrogens and (2) clarify the relationship between APOE genotypes and cognitive change in women without estrogens before the age of natural menopause we compared the cognitive performance of women with BRCA1/2 mutations who had undergone BSO with women with BRCA1/2 mutations who had not yet had BSO. In addition, because many women with BRCA1/2 mutations wait until they have had children to undergo BSO, women with BRCA1/2 mutations who have not undergone BSO are as many as 10 years younger than those who have had BSO. As age can have an influence on cognition, an age-matched control group was also included. We hypothesized that women without a primary source of endogenous estrogens will show deficits in verbal, spatial and working memory relative to women with intact ovaries. Test by test hypotheses are outlined in the methods section below. In addition, we hypothesized that the extent of changes will correlate with time since oophorectomy. Further, we hypothesized that women with E4 alleles will have decreased performance relative to women without an E4 allele.
Chapter 2: Methods

5 Participants

Participants were recruited from the Familial Breast Cancer Research Unit at Women’s College Research Institute (WCRI) in Toronto. The unit, headed by Dr. Steven Narod, screens men and women in the Greater Toronto Area for the presence of BRCA1 and BRCA2 mutations. Women who test positive for one or both of these mutations are subsequently counseled about their lifetime risk of breast and ovarian cancer and informed of BSO as an effective prophylaxis.

In addition, women without BRCA1 or BRCA2 mutations (or who have never been tested) were recruited through word of mouth with friends and colleagues as well as through and posters at the University of Toronto.

All women included in the study were between the ages of 30 and 51, had no medical history of depression or achieved a score higher than 17 on the CES Depression scale (CES-D; Radloff, 1977).

5.1 BSO Group

The BSO group consisted of 9 women with BRCA1/2 mutations who were also between 6 months and 8 years post-oophorectomy. As these women had not yet undergone natural menopause at the time of their elected surgery, BSO resulted in surgical menopause. At the time of testing women with BSO were not taking selective estrogen receptor modulators (SERMs) or aromatase inhibitors (AIs). Wherever possible, women who were not taking ERT were recruited.

5.2 BRCA Control Group

The BRCA control group consisted of 4 women who were diagnosed with a BRCA1/2 mutation and who have not had a BSO. At the time of testing women in this group were premenopausal, not on SERMs, AIs or any form of hormonal therapy. Wherever possible, women who were not on hormonal birth control were recruited.
5.3 Age Control Group

The Age control group consisted of 8 women between the ages of 40 and 50 who did not have a BRCA1/2 mutation or had never been tested. At the time of testing women in this group were pre or perimenopausal and were not taking any hormonal birth control or HRT.

6 Study Design

Women in the BSO and BRCA control groups were identified through medical records at the Familial Breast Cancer Unit. Women were selected who met the requirements for age, time since oophorectomy (6 months to eight years) and medications (where recorded). Of the three hundred women in the Familial Breast Cancer Unit, forty-nine women met the study requirements. Introduction letters describing the study were mailed to potential participants. Within two weeks a follow-up phone call was made to provide further information and when desired, schedule an appointment. Of the forty-nine women contacted, nineteen women declined to participate or were not eligible to participate and seventeen did not respond to recruitment phone calls or letters. In total fourteen women were recruited for this study, one of whom is scheduled for testing later in the year. Study appointments for women from the Familial Cancer Unit took place at WCRI, 790 Bay St.

Women in the Age control group were recruited through word of mouth and on campus posters. In either case an information sheet describing the experiment was read or emailed to potential participants. Follow-up communication was made to provide further information and when desired, schedule an appointment, which took place at Sidney Smith, 100 St. George St.

All women were scheduled for sessions in the morning to optimize circadian rhythm performance (Hasher et al., 2005). Participants were greeted by the experimenter and taken to a quiet room for testing. They were given an information sheet on the study and a consent form to sign. Additionally, a detailed form of pertinent medical history was filled out by each participant. Following consent and history, 10 cognitive task and one mood measure were administered in the following order (approximate administration time included):
This order was chosen to minimize overlap between tasks in the same domain. For example, one verbal memory task did not immediately follow another verbal memory task. In addition, tasks with a delayed condition were separated to reduce cognitive strain.

Following neuropsychological testing, participants were asked to provide a urine sample so that estrogen and progesterone metabolites: Estrone glucuronide (E1G) and Pregnanediol glucuronide (PdG) concentrations could be measured. In addition, a saliva sample was collected to be analyzed for APOE genotype. The clinical and experimental tests used in the battery are outlined below.

### 6.1 Demographic Tasks

**Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977)**

The CES-D is a short self-report scale that assesses mood and depression. The scale has 20 questions, on a four-point Likert scale, which ask respondents to indicate how often they felt a given statement was true during the last week, including the day of testing. Each question is scored 0, 1, 2 or 3. A final score out of 60 was obtained. Participants scoring higher than 17 were not be included in the study and were referred to counseling.

**North American Adult Reading Test (NAART; Strauss et al., 2006)**

The NAART consists of 61 irregularly spelled words. Because proper pronunciations of irregular words require previous exposure to these words, the NAART is highly correlated with education and serves as a quick assessment of intelligence. Number of errors made was used to calculate an estimate of full scale IQ (for equation, see Strauss et al., 2006). We hypothesized that IQ will not vary between groups.
6.2 Global Cognition

Logical Memory (LM; From Wechsler Memory Scale; Wechsler and Stone, 1945)
In LM participants are read two short stories and asked to repeat as much of each story as they can remember, both immediately afterwards and then again after a 20 minute delay. Items recalled from the two stories were tabulated and added together using gist scoring (Schwartz & Ivnik, 1980) for both the immediate and the delayed condition. We hypothesized that the BSO group would recall fewer story details than both Control groups in both the Immediate and Delayed recall conditions. In addition we hypothesized that the E4 group will recall fewer story details than the non-E4 group in the both the Immediate and Delayed recall conditions.

Semantic and Phonemic Fluency (Strauss et al., 2006; Troyer et al, 1997)
Participants are asked to name as many animals as they can in one minute. The procedure is repeated for words that begin with the letter “F.” Scoring was completed based on Troyer et al., 1997). For both semantic and phonemic fluency three variables were obtained: Total, Cluster size and Switches. Total denotes the total number of words given, excluding errors and repetitions. Clustering, or the production of words within the same semantic or phonemic subcategory, is related to temporal lobe functioning. Clusters were tabulated counting from the second word of a cluster: groups of a single word are scored as zero, groups of two words are scored as one and so on. A mean cluster size was then calculated. Switching, or shifting between clusters is related to frontal functioning (Troyer, 2000). Switches were calculated by counting the number of transitions between clusters. We hypothesized that the BSO group will have lower Semantic total scores than both Control groups. In addition Semantic and Phonemic cluster size will be smaller for the BSO group than for the Control groups. Phonemic total score and Semantic and Phonemic switches will not differ between groups. In addition we hypothesized that the E4 group would have lower Semantic total scores and smaller Semantic and Phonemic cluster size and than the non-E4 group.

6.3 Hippocampus

Rey Auditory Verbal Learning Test (RAVLT; Strauss et al., 2006)
The RAVLT consists of 15 words (List A) read aloud for 5 consecutive trials. Following each trial, participants are asked to list all words that they remember. Following the fifth trial, an interference list of 15 words (List B) is presented, followed by a free-recall test of that list.
Participants are then asked to list all the words they can remember from List A (short delay recall). Items recalled were tabulated for the 5 learning trials (A1-5), the interference list (Trial B) and the short delay recall (Trial A6). In addition, Retroactive interference (Best learning trial – Trial A6) and Proactive interference (Trial A1 – Trial A6) were calculated (Vakil & Blanchstein, 1994). Primacy score was tabulated as the number of times the first 3 words in the series were recalled across the 5 learning trials. Recency score was tabulated using the final 3 words (Vakil & Blanchstein, 1994). We hypothesized that the BSO group would recall fewer items across the 5 learning trials than both Control groups. In addition the BSO group would have greater Retroactive Interference than both Control groups, and smaller Recency scores. Scores on Proactive interference and Primacy scores will not differ between groups. In addition we hypothesized that the E4 group will recall fewer items across the 5 learning trials than the non-E4 group and in addition have greater Retroactive Interference scores.

**Remember/Know (Tulving, 1985)**

This test is given on a computer in two sessions. In the first session participants are instructed to learn a list of 60 words by assigning each word a pleasantness rating on a scale of 1 to 5. After a delay (20 minutes) the second session is administered in which participants are presented with a recognition task of 120 words (60 old and 60 new). For each word participants are instructed to 1) indicate if the word is old or new 2) indicate if the word was “Remembered” (remembering the event of encoding) “Known” (knowing the word was on the list by not remembering the event of encoding) or “Guessed” and 3) rate their confidence in their judgment on a scale of 1 to 5. Scores were calculated for Items correct (number of items correctly recognized as old or new /120), Hits (proportion of old items correctly identified as old), Proportion remembered (proportion of hits identified as being ‘remembered’), Proportion familiar (proportion of hits that were ‘familiar,’ for equation, see Yonelinas & Jacoby, 1995) and False alarms (number of new items incorrectly identified as old). We hypothesized that the BSO group would have lower Items correct and Hits than both Control groups. In addition, as time since oophorectomy increases, Proportion remembered will decrease and Proportion familiar will increase. Further, we hypothesized that the E4 group would have lower items correct and lower Hits than the non-E4 group. Scores on False alarms will not differ between groups.
Object Placement Task (Smith & Milner, 1981; Crane & Milner, 2005)

In the Object Placement Task participants are shown a board with 16 pictures of objects attached in a random spatial array. First participants are asked to name the 16 pictures. They are then asked to study the spatial arrangement of the objects. In total, participants are given 1 minute to study the board. They are then asked to reproduce the placement of the objects on a new, empty board both immediately and after a 20 minute delay. In order to obtain scores of Immediate and Delayed object displacement, we measured the x and y coordinates of each object and subtracted the original coordinates from these values; then an average of the 16 displacement values was taken. As values of displacement were taken, lower scores indicate better memory of the spatial array. We hypothesized that the BSO group would have higher Immediate and Delayed object displacement scores than both Controls groups. In addition we hypothesized that the E4 group would have higher Immediate and Delayed object displacement scores than the non-E4 group.

6.4 Frontal Cortex

Spatial Working Memory Task (SPWM; Duff & Hampson, 2001)

Participants are seated in front of a 4x5 array of doors. Underneath the doors there are 10 pairs of coloured dots. Participants are asked to find all 10 pairs of matching dots in as few moves as possible, by opening only two flaps at a time. Participants complete two trials. Number of working memory errors (reselecting locations that have already been searched, or revisiting a matched pair) were tabulated for Trial 1, Trial 2, and both trials combined (Total error). In addition, total time to completion for each trial was recorded and a Total time value was calculated (in seconds). We hypothesized that the BSO would have higher error scores than both Control groups on Trial 1 errors, Trial 2 errors, Total errors as well as longer Total times. In addition we hypothesized that the E4 group would have higher error scores than the non-E4 group on Trial 1 errors, Trial 2 errors, Total errors as well as longer Total times.

Digit Ordering Task (DOT; Petrides et al., 1993)

Participants are asked to say the numbers between 1 and 10 in a random order without repeating or omitting digits. Total errors were tabulated by summing the total number of omission or repetition errors over 10 trials. Average time to completion of each trial was calculated as well. We hypothesized that the BSO group would have higher Total errors and longer Average times
than both Control groups. In addition, we hypothesized that the E4 group would have higher Total errors and longer Average times than non-E4 group.

_Digit Span_ (WMS; Wechsler and Stone, 1945)

In Digits Forward participants are asked to repeat sequences of numbers of increasing length that are verbally presented by the experimenter. In Digits Backward participants must repeat each number sequence in the reverse order of its original presentation. While forward span only requires passive retention, backward span requires a manipulation of the digits in mind. The score on both scales was the maximum number of digits repeated correctly. In accordance with the theory that estrogen contributes to WM specifically by influencing the active manipulation element (Duff & Hampson, 2000) we hypothesized that the BSO would score lower on Digit span backward than both Control groups but that there will be no differences between groups in Digit span forward. In addition we hypothesized that the E4 group would score lower than the non-E4 group on Digit span backward but not Digit span forward.

_Corsi Block Tapping Task_ (Kessels et al., 2000)

The task uses a set of 9 identical blocks affixed to a board in a random array. Participants observe as the examiner taps out progressively longer sequences on the array of blocks. Immediately afterwards participants reproduce each sequence. Corsi block span was the length of the last successfully completely sequence. As the Corsi block tapping measures passive spatial span and not an active manipulation component we hypothesized that Corsi block spans will not differ between groups.

7  Statistical Analysis

Preliminary analysis identified outliers in the data set, which were replaced by a value equal to 2 standard deviations above the mean. This method was chosen due to small study sample size (Field, 2005). All skewed distributions were corrected by reducing outliers. Differences between groups were analyzed using one-way ANOVAs with group (BSO, Age control, BRCA control) as the main factor. Preplanned contrasts comparing: 1) women with BSO to women without BSO (BSO group to both Control groups) and 2) the two control groups (Age control group to BRCA control group) were included. Post-hoc comparisons using LSD pairwise comparisons
were included when ANOVAs or contrasts were significant. Non-parametric tests (Kruskal-Wallis and Mann Whitney) were used where homogeneity of variance could not be assumed. As age and education have an effect on performance on many cognitive tasks (Strauss et al., 2006), multiple regressions models were included to measure the role of covariates. Multiple regression models used the predictors Age, Years of education and BSO status (had or did not have BSO). In order to assess the direct impact of hormone levels on cognitive performance, scores were correlated with E1G and PdG concentrations on all tasks. Further, in order to assess the impact of time without ovaries, correlation analyses were made between test scores and time since oophorectomy on women in the BSO group. For individual scores, please see Appendix A.

As three women in the BSO group were taking ERT (two on 17beta-estradiol skin patches, one on CEE taken orally) all analyses were re-run excluding woman on ERT.
Chapter 3: Results

In total, 21 participants completed this study. One participant scored above 17 on the CES-D and was excluded from all further analyses. A second participant did not have English as her first language; only non-verbal tasks (SPWM, OPT, Corsi) tasks were included for this participant. A third participant in the BRCA control group was taking oral hormonal contraceptives; as she was tested during menses when pills contain no hormones (sugar pills are taken) this participant was kept in all analyses.

8 Demographics

Means and standard deviations for demographic data can be found in Table 1. An ANOVA revealed a main effect of group \( F(2,19) = 13.319, p<0.001 \). Post-hoc tests showed that BRCA control group was significantly younger than the BSO group \( p<0.001 \) and the Age control group \( p<0.001 \) but BSO and Age control groups did not significantly differ in Age \( p=0.946 \). No significant effects of group were found on Body Mass Index \( F(2,19)=0.258, p=0.775 \); Years of Education \( F(2,19)=1.350, p=0.286 \) and IQ \( F(2,18)=0.020 p=0.980 \) (as estimated by the North American Adult Reading Test; NAART).

Table 1. Demographic data: Means and standard deviations

<table>
<thead>
<tr>
<th></th>
<th>BSO (N=8)</th>
<th>Age Control (N=8)</th>
<th>BRCA Control (N=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Age</td>
<td>44.5 (5.043)</td>
<td>44.53 (2.560)</td>
<td>34.00 (1.155)</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>23.06 (2.783)</td>
<td>23.95 (4.435)</td>
<td>22.55 (1.630)</td>
</tr>
<tr>
<td>Education (in Years)</td>
<td>16.75 (1.669)</td>
<td>18.13 (1.533)</td>
<td>16.75 (2.630)</td>
</tr>
<tr>
<td>IQ (as estimated by NAART)</td>
<td>110.25 (7.340)</td>
<td>110.97 (6.477)</td>
<td>110.445 (7.437)</td>
</tr>
</tbody>
</table>
9 Hormone Levels

Means and standard deviations for E1G and PdG concentrations can be found in Table 2. An ANOVA revealed no significant effect of group on E1G concentration $F(2,17)=1.069$, $p=0.365$. Preplanned contrasts revealed no significant differences between the BSO group and both Control groups in E1G concentration $t(17)=-1.449$, $p=0.165$. As equal variances were not assumed on PdG concentration $p=0.006$, a Kruskal-Wallis test was used and revealed a significant effect of group on PdG concentration $H(2)=6.096$, $p=0.041$. Preplanned contrasts revealed that the BSO group had significantly lower PdG concentration than both Control groups $U=16.00$, $p=0.012$. There were no significant differences between the Age and BRCA control groups on E1G or PdG concentrations.

Table 2. Hormone levels: E1G and PdG concentrations

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>E1G</td>
<td>11.915 (12.224)</td>
<td>20.365 (11.844)</td>
<td>21.913 (17.737)</td>
</tr>
<tr>
<td>PdG*</td>
<td>0.221 (0.166)</td>
<td>2.147 (2.972)</td>
<td>1.295 (1.303)</td>
</tr>
</tbody>
</table>

* BSO< Controls $p<.05$

Excluding women on ERT, Kruskal-Wallis tests revealed a significant effect of group on E1G concentration $H(2)=5.884$, $p=0.044$; and a marginally significant effect of group on PdG concentration $H(2)=4.900$, $p=0.082$. Preplanned contrasts revealed that the BSO had significantly lower E1G concentration than both Control groups $U=7.000$, $p=.014$. An ANOVA revealed no significant effect of group on PdG concentration $F(2,14)=0.920$, $p=0.421$. Preplanned contrasts revealed no significant difference between PdG concentration in the BSO group as compared to the Control groups $t(14)=-0.970$, $p=0.349$. 
Means and standard deviations for women with BSO taking and not taking ERT can be found in Table 3. Not surprisingly, a Mann-Whitney test revealed that women with BSO on ERT had significantly higher E1G concentrations than women with BSO not on ERT U=0.000, p=0.036. An Independent t-test found no significant difference between PdG concentrations in women with BSO taking ERT versus those not taking ERT t(6)=0.569, p=0.590.

Table 3. Hormone levels: Women with BSO

<table>
<thead>
<tr>
<th></th>
<th>ERT (N=3)</th>
<th>No ERT (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M SD</td>
<td>21.303 (16.850)</td>
<td>6.282 (3.712)</td>
</tr>
<tr>
<td>E1G*</td>
<td>0.267 (0.271)</td>
<td>0.194 (0.096)</td>
</tr>
</tbody>
</table>

*ERT> No ERT p<.05

10 Global Cognition

10.1 Logical Memory (LM)

Means and standard deviations of LM can be found in Table 4. There was no significant effect of group on Immediate recall F(2,16)=2.512, p=0.113. Preplanned contrasts revealed that the BSO group recalled marginally significantly fewer story details than both Control groups t(16)=-1.770, p=0.096, but the Age and BRCA control groups did not significantly differ t(16)=1.032, p=0.317. Post hoc analyses showed that the BSO group recalled significantly fewer story details than Age control group p=.040 but not than the BRCA control group p=0.415. There was no significant effect of group on Delayed recall F(2,16)=1.393, p=0.277. Preplanned contrasts between the BSO group and the Control groups as well as between the Age and BRCA control groups were not significant p=0.171, p=0.567.
Table 4. Logical Memory: Means and standard deviation

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Immediate Recall*</td>
<td>13.563 (3.698)</td>
<td>20.492 (7.728)</td>
<td>16.625 (6.25)</td>
</tr>
<tr>
<td>Delayed Recall</td>
<td>10.563 (3.201)</td>
<td>16.117 (9.002)</td>
<td>13.75 (6.062)</td>
</tr>
</tbody>
</table>

*BSO<Age control; p<.05; BSO<Controls p<.1

Age, Years of education and BSO status were entered in to a regression analysis with Immediate recall as the dependant variable. The overall model was significant $F(3,15)=8.446$, $p=0.002$, $R^2=0.622$. Years of Education contributed significantly to the model $t(15)=3.943$, $p=0.001$ while BSO status contributed marginally significantly $t(15)=1.901$, $p=0.077$. Age did not contribute significantly to the model $t(15)=0.877$, $p=0.394$. The same regression analysis was run with Delayed recall as the dependant variable; the overall model was not significant $F(3,15)=3.781$, $p=0.033$, $R^2=0.431$. Years of education contributed significantly $t(15)=2.646$, $p=0.018$ but BSO status and Age did not $t(15)=1.324$, $p=0.205$; $t(15)=0.686$, $p=0.503$.

A logistic regression analysis was run using a value two standard deviations below norm means (Abikoff et al., 1987) as the impaired versus not-impaired cut point. BSO status was entered into the model with Immediate recall as the dependent variable. The overall model was not significant $\chi^2 (1)=0.177$, nor was BSO status a significant predictor $p=0.192$. Similarly, when the model was rerun with Delayed recall as the dependent variable, the model was not significant $\chi^2 (1)=0.411$, and BSO status was not a significant predictor $p=0.417$.

Correlations between E1G concentration and Immediate and Delayed recall were not significant $r(17)=-0.237$, $p=0.328$; $r(17)=-0.258$, $p=0.286$. No significant correlations were found between PdG concentration and Immediate or Delayed recall $r(17)=0.045$, $p=0.854$; $r(17)=-0.002$, $p=0.994$. Time since oophorectomy did not significantly correlate with Immediate or Delayed recall $r(6)=0.065$, $p=0.878$; $r(6)=-1.80$, $p=0.669$. 
Excluding women on ERT, there was no significant effect of group on Immediate recall $F(2,13)=1.822, p=0.201$. Preplanned contrasts comparing the BSO group to both Control groups was no longer significant $t(13)=-1.485, p=1.61$. A regression analysis was rerun with Immediate recall as the dependent variable and Age, Years of education and BSO status as the predictor variables. While the overall model was still significant $F(3,12)=6.652, p=0.007,R^2=0.624$ and Years of education remained a significant predictor $t(13)=3.501, p=0.004$, but BSO status was no longer a significant predictor $t(13)=0.986, p=0.344$. Age remained non-significant $t(13)=0.442, p=0.666$. All other analyses remained the same (see Appendix B).

10.2 Logical Memory (LM)

Means and standard deviations for Semantic and Phonemic Fluency are displayed in Table 5. A Kruskal-Willis test revealed a marginally significant effect of group on Semantic cluster size $H(2)=5.116, p=0.072$. Preplanned contrasts revealed a marginally significant difference between the BSO group and both Control groups on Semantic cluster size $U=23.500, p=0.091$ but no significant difference between cluster sizes for Age and BRCA control groups. One-way ANOVAs revealed no significant effects of group on Semantic total $F(2,16)=1.913, p=0.329$ or Semantic switches $F(2,16)=0.912, p=0.857$. Preplanned contracts found no differences between the BSO and both Control groups on Semantic total or Semantic switches $t(16)=1.199, p=0.248$; $t(16)=0.181, p=0.859$. No significant differences were seen between Age and BRCA control groups.

No significant effects of group were found on Phonemic fluency total $F(2,16)=0.849, p=0.446$, Phonemic cluster size $F(2,16)=1.629, p=0.227$ or Phonemic switches $F(2,16)=1.529, p=0.856$. Preplanned contrasts found no differences between the BSO group and both Control groups on Phonemic total, cluster size and switches $t(16)=.951, p=.356$; $t(16)=1.420, p=0.175$; $t(16)=0.351, p=0.730$. In addition no significant differences were seen between Age and BRCA control groups $t(16)=1.048, p=0.310$; $t(16)=1.345, p=0.175$; $t(16)=0.493, p=0.629$. 
Table 5. Phonemic and Semantic Fluency: Means and standard deviations

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Semantic Total</td>
<td>23.30 (5.787)</td>
<td>19.00 (5.099)</td>
<td>21.50 (4.933)</td>
</tr>
<tr>
<td>Cluster size*</td>
<td>1.249 (0.380)</td>
<td>0.775 (0.307)</td>
<td>1.278 (0.726)</td>
</tr>
<tr>
<td>Switches</td>
<td>10.13 (2.295)</td>
<td>10.34 (2.936)</td>
<td>9.50 (1.291)</td>
</tr>
<tr>
<td>Phonemic Total</td>
<td>17.88 (3.871)</td>
<td>17.43 (4.077)</td>
<td>15.00 (2.160)</td>
</tr>
<tr>
<td>Cluster size</td>
<td>0.526 (0.296)</td>
<td>0.448 (0.395)</td>
<td>0.178 (0.144)</td>
</tr>
<tr>
<td>Switches</td>
<td>14.025 (4.395)</td>
<td>14.429 (4.597)</td>
<td>11.75 (0.975)</td>
</tr>
</tbody>
</table>

*BSO>Controls, p<.1

There was a significant negative correlation between E1G concentration and both Semantic cluster size \( r(17) = -0.526, p = 0.021 \) and Phonemic cluster size \( r(17) = -0.466, p = 0.044 \) (see Figure 1). No significant correlations were between E1G concentration and Semantic total \( r(17) = -0.305, p = 0.204 \); Semantic switches \( r(17) = 0.224, p = 0.356 \); Phonemic total \( r(17) = -0.305, p = 0.204 \) or Phonemic switches \( r(17) = 0.125, p = 0.609 \). No significant correlations were found between PdG concentration and Semantic or Phonemic total cluster sizes or switches. Time since oophorectomy was not significantly correlated with Semantic total \( r(6) = -0.087, p = 0.838 \); Semantic cluster size \( r(6) = -0.168, p = 0.691 \); Semantic switches \( r(6) = -0.132, p = 0.756 \); Phonemic total \( r(6) = 0.499, p = 0.208 \); Phonemic cluster size \( r(6) = -0.013, p = 0.975 \) or Phonemic switches \( r(6) = -0.552, p = 0.156 \).
Excluding women taking ERT there was still no significant effect of group on Semantic total $F(2,13)=2.257$, $p=0.144$. However, preplanned contrasts comparing the BSO group to both Control groups revealed that the BSO group had marginally significantly higher Semantic total than both Control groups $t(13)=1.850$, $p=0.087$ (means and standard deviations in Table 6).

While ANOVAs for phonemic fluency variables remained not significant when women with ERT were excluded (see Appendix B), preplanned contrasts revealed that the BSO group had marginally significantly larger Phonemic cluster sizes than both Control groups $t(13)=1.798$, $p=0.095$. Post hoc analyses show that there is a marginally significant difference in phonemic cluster size between BSO and BRCA control groups $p=0.056$ but not between BSO and Age control groups $p=0.357$. Excluding women with ERT there is a significant negative correlation between Time since oophorectomy and phonemic fluency total $r(3)=-0.951$, $p=0.013$. All other analyses remained the same (see Appendix B).
Table 6. Phonemic and Semantic Fluency Excluding women on ERT: Means and SD

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Semantic Total*</td>
<td>25.68 (6.048)</td>
<td>19.00 (5.099)</td>
<td>21.50 (4.933)</td>
</tr>
<tr>
<td>Cluster size*</td>
<td>1.389 (0.367)</td>
<td>0.775 (0.307)</td>
<td>1.278 (0.726)</td>
</tr>
<tr>
<td>Switches</td>
<td>10.40 (2.510)</td>
<td>10.34 (2.936)</td>
<td>9.50 (1.291)</td>
</tr>
<tr>
<td>Phonemic Total</td>
<td>18.20 (3.633)</td>
<td>17.43 (4.077)</td>
<td>15.00 (2.160)</td>
</tr>
<tr>
<td>Cluster size**</td>
<td>0.626 (0.284)</td>
<td>0.448 (0.395)</td>
<td>0.178 (0.144)</td>
</tr>
<tr>
<td>Switches</td>
<td>11.80 (3.114)</td>
<td>14.429 (4.597)</td>
<td>11.75 (0.975)</td>
</tr>
</tbody>
</table>

* BSO> Controls, p<.1; * BSO>Control, p<.1 **BSO>Controls, p<.1; BSO>BRCA, p<.1

11 Hippocampus

11.1 Rey Auditory Verbal Learning Test (RAVLT)

Analysis of the RAVLT learning trials revealed no significant effects of group on Trials A1-5 (see Figure 2). In addition there was no significant effects of group on the interference list (Trial B), F(2,16)=0.100, p=0.906 or on short delay recall (Trial A6) F(2,16)=2.456, p=0.117. Preplanned contrasts reveal no significant differences between the BSO group and both Control groups on Trials A1-5, B or A6. However, the BRCA control group recalled significantly more items than on the short delay recall (Trial A6) than the Age control group t(16)=−2.216, p=0.042.
Means and standard deviations for Retroactive interference, Proactive interference, Primacy and Recency scores can be found in Table 7. A Kruskal-Wallis test revealed no significant effect of group on Retroactive interference (Best learning trial – Trial A6) $H(2)=4.361$, $p=0.112$. An ANOVA revealed no significant effect of group on Proactive interference (Trial A1 – Trial B) $F(2,16)=0.806$, $p=0.464$. Preplanned contrasts showed no difference between the BSO group and both Control groups on either measure of interference $U=38.500$, $p=0.657$; $t(16)=0.561$, $p=0.583$. However the Age control group showed marginally significantly more Retroactive interference than the BRCA control group $U=5.000$, $p=0.094$. To see if the Age control groups, differed from both BRCA groups (BSO and BRCA control group) an additional contrast was run on which the Age control group showed significantly more Retroactive interference than both BRCA groups $U=21.000$, $p=0.076$.

There was a marginally significant effect of group on Primacy score $F(2,16)=3.139$, $p=0.071$ but not on Recency score $F(2,16)=0.307$, $p=0.740$. Preplanned contrasts show a marginally significant difference in Primacy score the BSO group and both Control groups $t(16)=−1.995$, $p=0.063$ as well as between Age control and BRCA control groups $t(16)=−1.851$, $p=0.083$. There was no
difference between the BSO group and both Control groups on Recency score \( t(16)=1.468, p=0.506 \).

Table 7. RAVLT: Means and standard deviations

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Retroactive Interference*</td>
<td>1.50 (1.069)</td>
<td>2.90 (2.006)</td>
<td>0.75 (0.500)</td>
</tr>
<tr>
<td>Proactive Interference</td>
<td>1.38 (2.200)</td>
<td>2.71 (2.752)</td>
<td>1.25 (1.258)</td>
</tr>
<tr>
<td>Primacy score**</td>
<td>11.75 (1.488)</td>
<td>12.29 (1.496)</td>
<td>14.00 (1.414)</td>
</tr>
<tr>
<td>Recency Score</td>
<td>12.38 (1.302)</td>
<td>13.00 (1.732)</td>
<td>12.75 (1.708)</td>
</tr>
</tbody>
</table>

*Age control>BRCA control \( p<.1 \); Age control > BRCA groups \( p<.1 \).

**BSO< Controls, \( p<.1 \); Age Control< BRCA Control, \( p<.1 \)

There were no significant correlations between E1G or PdG concentrations and Trials A1-A5, Trial B or Trial A6. Further there were no significant correlations between E1G or PdG concentrations and Retroactive interference, Proactive interference, Primacy or Recency scores. Time since oophorectomy was significantly negatively correlated with number of items recalled on Trial A1 \( r(6)=-0.878, p=0.004 \); Trial A4 \( r(6)=-0.653, p=0.079 \); Trial A5 \( r(6)=-0.755, p=0.030 \); and Trial A6 \( r(6)=-0.778, p=0.023 \) (see Figure 3). In addition there is a significant negative correlation between Time since oophorectomy and Primacy score \( r(6)=-0.833, p=0.010 \).
Excluding women taking ERT, there was a significant effect of group on Primacy score $F(2,13)=4.035$, $p=0.043$. Preplanned contrasts show a significant difference between the BSO group both Control groups on Primacy score $t(13)=-2.414$, $p=0.031$. Excluding women on ERT, there was a marginally significant negative correlation between Time since oophorectomy and Items recalled on Trial A1 $r(3)=-0.863$, $p=0.060$ and Trial A5 $r(3)=-0.868$, $p=0.057$; but correlations between time since oophorectomy and Trial A4 $r(3)=-0.674$, $p=0.212$ and A6 $r(3)=-0.767$, $p=0.131$ were no longer significant. There was still a significant negative correlation between Time since oophorectomy and Primacy score $r(3)=-0.892$, $p=0.042$. All other analyses remained the same (see Appendix B).

### 11.2 Remember/Know Task (R/K)

Three participants did not complete the R/K task. One set of data was lost as a result of computer difficulties, one participant could not complete the task due to time constraints on the testing session and one participant did not comply with the task instructions. Means and standard deviations are displayed in Table 8. ANOVAs revealed no significant effects of group on Items correct $F(2,13)=0.088$, $p=0.916$; Hits $F(2,13)=0.458$, $p=0.642$; Proportion remembered $F(2,13)=0.899$, $p=0.431$; Proportion familiar $F(2,13)=0.840$, $p=0.454$ or False alarms $F(2,13)=0.049$, $p=0.952$. Preplanned contrasts revealed no significant differences between the
BSO group and both Control groups or between Age and BRCA control groups on any of the variables.

E1G and PdG concentrations were not significantly correlated with Items correct, Hits, Proportion remembered, Proportion familiar or False alarms. There was a significant negative correlation between Time since oophorectomy and Proportion remembered \( r(3)=-0.899, \) \( p=0.038. \) Excluding women taking ERT all analyses remained the same (see Appendix B). Time since oophorectomy correlations could not be re-run due to low sample size (n=2).

Table 8. Remember Know: Means and standard deviations

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Items correct</td>
<td>103.60 (7.570)</td>
<td>101.10 (11.743)</td>
<td>102.00 (9.764)</td>
</tr>
<tr>
<td>Hits</td>
<td>.91 (0.065)</td>
<td>.87 (0.094)</td>
<td>.91 (0.044)</td>
</tr>
<tr>
<td>Proportion Remembered</td>
<td>.72 (0.316)</td>
<td>.58 (0.182)</td>
<td>.74 (0.135)</td>
</tr>
<tr>
<td>Proportion Familiar</td>
<td>.68 (0.404)</td>
<td>.85 (0.127)</td>
<td>.88 (0.206)</td>
</tr>
<tr>
<td>False Alarms</td>
<td>.18 (0.086)</td>
<td>.17 (0.172)</td>
<td>.21 (0.179)</td>
</tr>
</tbody>
</table>

11.3 Object Placement Task (OPT)

Means and standard deviations of object displacement are displayed in Table 9. ANOVAs revealed no significant effect of group on the Immediate or Delayed object displacement \( F(2,17)=0.859, p=0.441; F(2,17)=0.313, p=0.736. \) Preplanned contrasts showed no differences between the BSO group and both Control groups on the Immediate or Delayed object displacement \( t(17)=-1.273, p=0.220; t(17)=-0.161, p=0.874. \) There were no significant differences between Age and BRCA control groups.

Years of education and BSO status were entered into a regression analysis with Immediate object displacement as the dependant variable. The overall model was significant \( F(2,17)=4.700, p=0.024, R^2=0.356. \) Years of education contributed significantly to the model \( p=0.017 \) as did
BSO status $p=0.045$. The same regression analysis was run with Delayed object displacement placement as the dependant variable; the overall model was not significant $F(2,17)=2.486$, $p=0.113$, $R^2=0.226$ and only Years of education contributed significantly to the model $p=0.042$.

Table 9. Object Placement Task: Means and standard deviations of object displacement

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M$</td>
<td>SD</td>
<td>$M$</td>
</tr>
<tr>
<td>Immediate object displacement</td>
<td>5.441 (0.874)</td>
<td>6.416 (1.887)</td>
<td>6.391(2.166)</td>
</tr>
<tr>
<td>Delayed object displacement</td>
<td>7.230 (1.678)</td>
<td>7.797 (2.141)</td>
<td>6.953(1.868)</td>
</tr>
</tbody>
</table>

There was a significant positive correlation between E1G concentration and Immediate object displacement $r(18)=0.635$, $p=0.003$ (see Figure 4). There were no significant correlations between E1G concentration and Delayed object displacement or between PdG concentration and Immediate or Delayed object displacement. There were no significant correlations between Time since oophorectomy and Immediate object displacement $r(18)=-0.532$, $p=0.174$; but there was a marginally significant negative relationship between time since oophorectomy and Delayed object displacement $r(18)=-0.667$, $p=0.071$.

Figure 4: Correlation between E1G concentration and Immediate object displacement
Excluding women on ERT, Years of education and BSO status were entered into a regression model with Immediate object displacement as the dependent variable. The overall model remained significant as did Years of education and BSO status (see Appendix B). The same regression analysis was run with Delayed object displacement as the dependent variable. The overall model was now marginally significant $F(2,14)=3.405$, $p=0.062$, $R^2=0.327$. Once again Years of education contributed significantly to the model $p=0.023$ but BSO status did not $p=0.190$. Excluding women on ERT, the correlation between Time since oophorectomy and Delayed object displacement was no longer significant $r(3)=-0.799$, $r=0.105$. All other analyses remained the same (see Appendix B).

12 Frontal Cortex

12.1 Spatial Working Memory Task (SPWM)

Means and standard deviations for the SPWM task are displayed in Table 10. ANOVAs revealed no significant effects of group on Trial 1 errors $F(2,17)=0.240$, $p=.790$; Trial 2 errors $F(2,17)=0.106$, $p=0.900$; Total errors $F(2,17)=0.288$, $p=0.753$ or Total time $F(2,17)=0.133$, $p=0.876$. Preplanned contrasts revealed no significant differences between the BSO group and both Control groups on Trial 1 errors $t(17)=-0.672$, $p=0.551$; Trial 2 errors $t(17)=-0.384$, $p=0.706$; Total errors $t(17)=-0.608$, $p=0.551$ or $t(17)=0.479$, $p=0.638$. In addition there were no significant differences between Age and BRCA Control groups.

Table 10. SPWM: Means and standard deviations

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Trial 1 Errors</td>
<td>33.25 (14.089)</td>
<td>38.14 (16.766)</td>
<td>38.00 (14.629)</td>
</tr>
<tr>
<td>Trial 2 Errors</td>
<td>45.50 (9.813)</td>
<td>49.05 (16.765)</td>
<td>47.50 (21.764)</td>
</tr>
<tr>
<td>Total Errors</td>
<td>78.63 (21.206)</td>
<td>89.40 (31.435)</td>
<td>84.00 (34.881)</td>
</tr>
<tr>
<td>Total Time (in seconds)</td>
<td>480.375 (166.850)</td>
<td>458.250 (179.543)</td>
<td>429.000 (111.029)</td>
</tr>
</tbody>
</table>
There were no significant correlations between E1G concentration and Trial 1 errors, Trial 2 errors, Total errors or Total time. There was a significant positive correlations between PdG concentration and Trial 2 errors $r(18)=0.622$, $p=0.003$ as well as between PdG concentration and Total errors $r(18)=0.527$, $p=0.017$ (see Figure 5). Correlations between PdG concentration and Trial errors and Total time were not significant $r(18)=0.195$, $p=0.411$; $r(18)=-0.232$, $p=0.324$. Correlations between Time since oophorectomy and Trial 1 errors, Trial 2 errors, Total errors, and Total time were not significant.

Figure 5: Correlation between PdG concentration and SPWM working memory errors

Results on the SPWM task did not differ when women on ERT were excluded (see Appendix B). However, women with BSO who were not taking ERT (mean=383.800) has significantly faster Total times than women who were taking ERT (mean=641.333) $t(6)=3.253$, $p=.017$. Further, when women on ERT were excluded there was a marginally significant positive correlation between E1G concentration and Trial 2 errors $r(15)=0.457$, $p=0.065$ as well as a marginally significant positive correlation between E1G concentration and Total errors $r(15)=0.454$, $p=0.067$. 
12.2 Digit Ordering Task (DOT)

Means and standard deviations for the DOT are displayed in Table 11. ANOVAs revealed no significant effect of group on Total errors F(2,16)=0.764, p=0.482 or on Average time F(2,16)=0.770, p=0.479. Preplanned contrasts revealed no significant differences between the BSO and both Control groups on Total errors t(16)=−0.511, p=0.616 or on Average time t(16)=−0.875, p=0.395. There were no significant differences between Age and BRCA control groups on Total errors t(16)=1.015, p=0.325 or Average time t(16)=−1.024, p=0.321.

There were no significant correlations between E1G or PdG concentrations and Total Errors r(17)=0.138, p=0.572; r(17)=0.055, p=0.824 or Average Time r(17)=0.133, p=0.588; r(17)=−0.133, p=0.587. There were no significant differences between Time since oophorectomy and Total errors r(17)=0.406, p=0.318 or Average time r(17)=−0.514, p=0.192. Excluding women on ERT did not change the outcome of analyses (see Appendix B).

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
</table>

12.3 Digit Span and Corsi Block Tapping Task

Means and standard deviations for Digit Span and Corsi block tapping are displayed in Table 12. There were no significant effects of group on Digit Span Forward F(2,16)=0.408, p=0.672; Backward H(2)=0.734, p=0.708; or Corsi Block Span F(2,17)=0.149, p=0.863. Preplanned contrasts between the BSO group and both Control groups revealed no significant differences on Digit Span Forward, Backward or Corsi Block Span. In addition there were no significant differences between Age and BRCA control groups.

There were no significant correlations between E1G or PdG concentration and Digit Span Forward, Backward and Corsi Block Span. In addition there were no significant correlations
between Time since oophorectomy and Digit Span Forward $r(6) = -0.460$, $p=0.251$; Backward $r(6) = -0.129$, $p=0.761$ and Corsi Block Span $r(6) = -0.075$, $p=0.860$. Excluding women on ERT did not change the outcome of analyses (see Appendix B).

Table 12. Digit Span and Corsi Block Tapping Task: Means and standard deviations

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Digit Span Forward</td>
<td>6.88 (1.246)</td>
<td>7.14 (0.900)</td>
<td>6.50 (1.291)</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>4.75 (1.389)</td>
<td>5.14 (1.574)</td>
<td>5.25 (0.500)</td>
</tr>
<tr>
<td>Corsi Block Span</td>
<td>6.00 (0.926)</td>
<td>6.00 (0.926)</td>
<td>6.31 (1.371)</td>
</tr>
</tbody>
</table>

13 APOE

It was not possible to complete APOE genotyping in time for this report. Possible influences of APOE are explored in the discussion.
Chapter 4: Discussion

Previous studies have shown that estrogens can have both positive and negative effects on cognition. The results of the present study show both of these outcomes as well as no effects depending on the task.

13.1 Global Cognition and Hippocampus

Similar to Phillips & Sherwin (1992) we found that women with BSO performed worse than Control groups on Logical Memory Immediate but not Delayed recall. This result is in agreement with the ERT literature that indicates that paragraph recall tasks like LM are the most consistently sensitive measure of the effects of ERT on verbal memory (Zec & Trivedi, 2002). While often considered within the HRT literature to be a test of verbal memory, factor analysis of WMS scales indicates that in addition to learning, LM Immediate recall has an attention component (Ernst et al., 1986). This is still true when the factor analysis model includes another verbal memory task, such as the RAVLT (Smith et al., 1992). Investigations of narrative comprehension suggest that the prefrontal cortex plays a role in recalling the temporal structure of narrative events (Zalla et al., 2002). Decreased LM performance coupled with no significant differences in RAVLT or R/K performance indicates that attention abilities may be related to estrogen levels. This hypothesis is supported by animal studies that show that E2 modulates attention performance in monkeys (Tinkler & Voytko, 2005). Further, menstrual cycle studies found improved performance on tasks of sustained attention in the luteal phase when estrogen and progesterone levels are high (Soliz-Oritz et al., 2004; Solis-Oritz & Corsi-Cabrera, 2008). As well, a number of HRT studies found women given HRT performed better on attention tasks compared to women not given HRT (Dumas et al., 2006; Ghidoni et al., 2006; Krug et al., 2006).

One proposed mechanism by which estrogen affects attention is via the cholinergic system by increasing activity of choline acetyltransferase and enhancing synaptic connectivity in the hippocampus and frontal lobe (Dumas et al., 2008; Lacalle et al., 2008; Simpkins et al., 1997). Imaging evidence also points to a role of the parietal cortex in ERT related attention improvement (Ghidoni et al., 2006; Stevens et al., 2005). In particular, treatment with CEE results in increased activation of the inferior parietal lobe during storage of verbal material, as is done in LM (Shaywitz et al., 1999). Further research into the role of the parietal lobe is needed to clarify the relationship between estrogens and attention.
Consistent with our findings, norms for LM indicate that years of education have a greater effect on Immediate and Delayed recall (gist scoring) than age (Abikoff et al., 1987). Norms for LM can be found in Tables 13. It is evident that means for all three groups are below norms on both Immediate and Delayed recall. This may be because we used the original version of the WMS from 1945 (in order to replicate the results seen in Phillips & Sherwin, 1992). Consequently, many of the turns of phrase and expressions used in the stories in LM are difficult for participants because they are no longer commonly used. As gist scoring requires individuals to recognize the meaning, but not necessarily precise wording of certain phrases, unfamiliar phrases may result in lower scores. In addition, these norms were published in 1987, and normative values for this scale may have changed in the years since. However, it is also clear that means for BSO group are the furthest from norms levels. While logistic regression models using BSO status to predict impairment were not significant, calculations of z-scores reveals that 4 of 8 women are 2 standard deviations below the mean for Immediate recall, reaching the criteria for impairment.

<table>
<thead>
<tr>
<th>Table 13. Logical Memory: Norms and Group Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate</td>
</tr>
<tr>
<td>_______</td>
</tr>
<tr>
<td>M</td>
</tr>
<tr>
<td>Norms 40-49</td>
</tr>
<tr>
<td>BSO</td>
</tr>
<tr>
<td>Age Control</td>
</tr>
<tr>
<td>Norms 30-39</td>
</tr>
<tr>
<td>BRCA Control</td>
</tr>
</tbody>
</table>

Source for norm values: Abikoff et al. (1987).

While there are no overall differences between groups on any individual trial on the RAVLT, correlation evidence from this study indicates that performance on the RAVLT, including short delay recall (Trial A6), decreases as time since oophorectomy increases. These results suggest a decrease in hippocampal function over time. It may be the case that decreased performance on
hippocampus related tasks do not appear until women have been without endogenous estrogen for a number of years. As 5 of the 8 women in the BSO group had their surgery within a year of testing, overall group differences may have not yet been manifest. Nappi et al., (1999) found a similar correlation between time since oophorectomy and performance on a word list task (Serial Learning Test). Further, results from epidemiological research of Rocca and colleagues (2007) suggest that age of oophorectomy (and by association, time spent without E2) is related to dementia risk. As short delay recall on the RAVLT has been found to be a significant predictor of AD (Tierney et al., 2005), decreasing performance on this task as time without endogenous E2 accumulates suggests that women with surgical menopause may be at increased risk for dementia.

In the present study Age controls showed marginally significantly greater retroactive interference on the RAVLT than BRCA controls. Retroactive interference is the “decremental effect of subsequent learning on retention of previously learning material” (Strauss et al., 2006, p.779). As the BSO group did not show different levels of retroactive interference than the Control groups, it cannot be said that estrogens are driving the differences between groups on this measure. As can be noted in Table 7, variance in the Age control group is larger than in the BSO and BRCA Control group. It may be the case that presence of women with APOE E4 is increasing variance of performance on this task (see below for more complete discussion). Higher Retroactive interference can also be accounted for by increased engagement with the interference list. As LM results indicate, women with BSO do not engage with new, non-repeated verbal information as well as women in the Control groups. However, no significant differences were found between groups on Trial B. In addition, Retroactive Interference in the Age control group was significantly higher than in both BRCA groups (BSO and BRCA control groups). This indicates that the BRCA1/2 mutations may be playing a role in performance on this task. Further research is needed to confirm this hypothesis.

In the present study there was an effect of group on Primacy score on the RAVLT. Past studies have shown that AD patients show reduced primacy effects (Tierney et al., 1994). Nappi et al., (1999) compared women with BSO to age-matched controls and found women with BSO recalled significantly fewer recency items on the Serial Learning Test, while finding no difference in primacy items. However, the Serial Learning Test uses ten different word lists whereas the RAVLT repeats the same list of words five times. In addition, we found that while
the BSO group had significantly lower primacy scores than both Control groups, the Age control
group also had lower primacy scores than the BRCA control group (see Table 7). This suggests
that age may play a role in recall of older list items. Removal of participants taking ERT
strengthens the effect.

Norms for RAVLT can be found in Table 14. Unlike the LM, RAVLT performance is more
sensitive to IQ than Years of education (Strauss et al., 2006). Norm groups from Geffen et al.,
(1990) have lower years of education but similar IQ to groups in the present study. As can be
seen group means are above norm values with a few exceptions: BRCA controls Trial A1 and
A2, which are within 0.2 standard deviations of the norm. This indicates that all groups are
performing within the normal range on this task.

Table 14. RAVLT: Norms and Group Scores

<table>
<thead>
<tr>
<th>Trial</th>
<th>Norm 40-49</th>
<th>BSO</th>
<th>Age Control</th>
<th>Norm 30-39</th>
<th>BRCA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>A1</td>
<td>6.8 (1.5)</td>
<td>7.5 (1.77)</td>
<td>8.57 (1.13)</td>
<td>8.0 (2.0)</td>
<td>7.75 (2.75)</td>
</tr>
<tr>
<td>A2</td>
<td>9.4 (1.5)</td>
<td>10.75 (1.49)</td>
<td>11.43 (2.07)</td>
<td>10.8 (2.1)</td>
<td>10.50 (2.65)</td>
</tr>
<tr>
<td>A3</td>
<td>11.4 (1.7)</td>
<td>12.88 (2.10)</td>
<td>12.29 (2.29)</td>
<td>11.5 (1.7)</td>
<td>13.25 (1.50)</td>
</tr>
<tr>
<td>A4</td>
<td>11.7 (2.1)</td>
<td>12.25 (2.38)</td>
<td>13.00 (2.00)</td>
<td>12.9 (1.3)</td>
<td>13.75 (1.50)</td>
</tr>
<tr>
<td>A5</td>
<td>12.8 (1.4)</td>
<td>13.41 (1.86)</td>
<td>13.57 (1.13)</td>
<td>12.7 (1.3)</td>
<td>14.00 (1.41)</td>
</tr>
<tr>
<td>B1</td>
<td>5.2 (1.3)</td>
<td>6.13 (2.03)</td>
<td>5.86 (2.48)</td>
<td>6.5 (1.5)</td>
<td>6.50 (2.52)</td>
</tr>
<tr>
<td>A6</td>
<td>11.1 (2.4)</td>
<td>12.13 (1.96)</td>
<td>11.14 (2.55)</td>
<td>12.1 (1.9)</td>
<td>14.00 (0.82)</td>
</tr>
</tbody>
</table>

Source for norm values: Geffen et al., (1990) based on a sample of women only, mean years of
education and IQ 11.7 years/113.3 for 40-499 group and 10.9 years/111.9 for 30-39 year old
group.

In the present study there were no significant differences between groups on the R/K task. This
suggests that women with BSO are not compensating for decreased hippocampal performance by
relying on familiarity processes. The R/K task is designed to measure the differing contributions
of recollection and familiarity to recognition memory. Recollection and familiarity are thought to use differing medial temporal lobe and frontal structures. Neuroimaging studies have shown that activity in the hippocampus, parahippocampus and anterior left prefrontal cortex correlates with recollection while activity in the perirhinal cortex as well as right and lateral prefrontal cortex correlates with familiarity (Cohn et al., 2009; Diana et al., 2007; Henson et al., 1999). As hippocampal engagement is determined by recollection of context (Cohn et al., 2009) we hypothesized that as time since oophorectomy increases, Proportion remembered will decrease and Proportion familiar will increase. In fact, there was a significant correlation between time since oophorectomy and Proportion remembered. However, due to three participants not completing the task, this correlation only included 5 participants (see Figure 6), which allowed a single participant who is 5 years post oophorectomy to drive the significance. Indeed, when the participant is removed, the correlation is no longer significant r(2)=0.021, p=0.979.

Figure 6: R/K: Correlation between Time since oophorectomy and Proportion remembered

In the present study there is a negative relationship between E1G levels and both Semantic and Phonemic cluster sizes. Further, when women on ERT were removed from analyses, the BSO group had higher Semantic fluency total as well as larger cluster sizes than both Control groups. As animal fluency is considered an important predictor of AD (Henry et al., 2004; Tierney et al., 2005) it is surprising to discover that when women taking ERT are removed from analyses, the BSO group has the highest scores on Semantic fluency. While decreased cluster size has been
found in AD patients (Troyer et al., 1998), another study suggests that decreased switching is predictive of AD (Raoux et al., 2008). Sex difference analyses of clustering and switching on fluency tasks show that women switch more often than men, whereas men produce larger cluster sizes (Weiss et al., 2006). This sex difference is consistent with our finding that low estrogen levels result in larger cluster sizes. Meta analyses reveal a small advantage for females on phonemic fluency but not semantic fluency. However, at least one norm study of clustering and switching failed to find a significant effect of sex (Troyer, 2000). As cluster size is related to temporal lobe functioning (Troyer, 2000), our finding of a negative correlation between E1G concentration and cluster size indicates the possibility of women with BSO using compensatory strategies in order to conserve or improve cognitive abilities.

While there were no significant differences between groups on OPT performance, regression analyses and correlational results suggest that individuals with lower levels of estrogens perform better on the OPT (less object displacement). Past studies suggest a similar relationship between estrogen levels and spatial abilities such that women perform bests on tests of spatial abilities such as mental rotation when estrogen levels are lowest (Hampson, 1995). While Silverman and colleagues (2007) propose a positive relationship between estrogens and object location memory, this hypothesis has never been tested aside from studies comparing males and females.

The Object Placement Task used in this study differs from Silverman and Eal’s Location Exchange task in a number of ways. While the OPT measures exact placement of items relative to a study boards, the Location-Exchange task presents participants with a stimulus array board displaying 25-30 objects. Participants are then shown a test array and asked to note which items have switched locations. As the OPT is concerned with absolute rather than relative location, it may be more closely related to spatial abilities that have in the past shown negative relationships with estrogen such as spatial visualization and metric positional construction (Hampson, 2008). Use of relative location scoring method on the OPT or a different spatial paradigm such as the Location-Exchange task might reveal poorer spatial performance in women with BSO. However, as OPT performance is sensitive to hippocampal functioning (Smith & Milner, 1981; Crane & Milner, 2005), a negative relationship between OPT performance spatial abilities and E1G concentration indicates that while some cognitive abilities decline in the absence of endogenous estrogens, others improve. Heister et al., (1989) found that functional asymmetries in verbal and non-verbal tasks are greatest when ovarian hormone levels are lowest, which is during menses.
They argue that these functional asymmetries, evident when hormone levels are low, show a more “male” pattern of lateralization. Similarly, Bayer & Hausmann (2009) found that HRT increased bilaterality in verbal and non-verbal tasks. The idea that ovarian hormones differentially affect verbal and non-verbal tasks indicates that while BSO may negatively impact verbal memory and attention, it may be beneficial for spatial abilities.

Taken together, these results suggest that estrogen deprivation affects multiple brain regions, with effects on the hippocampus increasing with time and that possible cognitive advantages emerge in spatial cognition and fluency clustering when hormone levels are low.

### 13.2 Frontal Cortex

As expected there were no difference in digit and spatial spans between groups. This is consistent with several HRT and surgical menopausal studies (Duff & Hampson, 2000, Phillips & Sherwin, 1992; Resnick & Maki, 2001). However, the present study found no significant differences in working memory errors on Digits Backward, SPWM and DOT. This is in contrast to past studies which have found the women on HRT perform better on tasks of working memory that include a manipulation component (Duff & Hampson, 2000; Krug et al., 2006). This difference in results may be due to a large range of normal performance on these tasks coupled with the low sample size in this study. However, in looking at correlations between hormonal status and performance on these tasks, results suggest that progesterone levels may play a role in SPWM performance. There was a significant positive correlation between PdG levels and Trial 2 errors as well as Total Errors. The direction of this relationship suggests that as PdG concentration increases so do working memory errors. This correlation contrasts with Duff and Hampson’s results (2000) showing that women taking HRT (estrogens with progesterone) had the fewest working memory errors, lower than women taking ERT or with no hormone treatment at all. However, differences between the HRT and ERT groups in this study were not significant. In contrast, progesterone has been found to decrease working and spatial memory in ovariectomized rats (Bimonte-Nelson et al., 2004; Braden et al., 2010) As well, in general, progesterone has been found to have antagonistic effects in other body systems (Chen et al., 1998; Crook & Stevenson, 1996). It is also possible that a single participant may drive the significance of this finding (see Figure 5) because when that participant is removed from the analysis, the correlation between PdG concentration and Trial 2 errors is no longer significant.
r(19)=0.388, p=0.101. However, the correlation between PdG concentration and Total errors remains marginally significant r(17)=0.407, p=0.084.

Analysis of our data with ERT users separated out indicates that there is still a negative relationship between estrogens and working memory performance on SPWM. A comparison of women with BSO who are and are not taking ERT reveals that women not taking ERT had significantly faster times to completion than women who were taking ERT. File et al., (2002) also compared surgically menopausal women also comparing women who were taking or not taking ERT for 10 years following surgery. They found that women who were not taking ERT performed significantly better on a frontal function (rule reversal) task. When women taking ERT are excluded from analyses, a marginally significant positive correlation between working memory errors and E1G concentration emerges. Taken together these results indicate that endogenous and synthetic ovarian hormones, estrogens and particularly progesterone, may have negative effects on working memory performance. Further, as discussed previously, estrogens have a negative relationship with many spatial abilities. Given the spatial nature of this working memory task, low estrogen levels may provide an advantage for women with BSO.

Taken together, these results suggest a negative relationship between ovarian hormones and working memory.

13.3 Variance with Groups

On many tasks in this study, including Logical Memory, RAVLT (Retroactive Interference), R/K, OPT, SPWM and DOT, the variance observed within each of the two control groups was greater than that in the BSO group (see Table 15). Though E1G concentrations in the BSO group are not uniformly low (see Table 2), all women with BSO, including those taking ERT no longer experience cyclic hormonal changes. It may be the case that in the absence of natural hormonal variation, cognitive variance is reduced.

Higher variance in the control groups may also be due to the presence of women with APOE genotype E4. Individuals with APOE E4 score lower on tests of cognition, particularly tests of verbal memory, response inhibition and cognitive flexibility (Bondi et al., 1995; Bondi et al., 1999; Wetter et al., 2005). Further, even when neuropsychological measures do not show significant differences between genetic groups, statistical and imaging analyses still show greater
heterogeneity of performance and greater magnitude in the extent of brain responses (Bondi et al., 2005; Wierenga et al., 2010). Unfortunately, APOE analysis could not be included in this study, but future analyses of APOE genotype may account for some of the variance observed in control groups.

<table>
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<tr>
<th>Task</th>
<th>BSO</th>
<th>Age Control</th>
<th>BRCA Control</th>
<th>p-value*</th>
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<td>10.246</td>
<td>81.027</td>
<td>36.748</td>
<td>.066</td>
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<td>RAVLT Retroactive</td>
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<td>4.024</td>
<td>0.250</td>
<td>.027</td>
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<tr>
<td>R/K Items Correct</td>
<td>60.062</td>
<td>137.898</td>
<td>95.336</td>
<td>.568</td>
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<td>OPT Immediate</td>
<td>0.764</td>
<td>3.562</td>
<td>4.477</td>
<td>.276</td>
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<td>OPT Delayed</td>
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<td>3.489</td>
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<td>SPWM Total Errors</td>
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<td>988.159</td>
<td>1216.684</td>
<td>.422</td>
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<tr>
<td>DOT Total Errors</td>
<td>22.743</td>
<td>46.145</td>
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<td>.423</td>
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* using Levene’s Test

13.4 Limitations of the Study

The present study has a number of limitations. Perhaps most importantly, all groups have a low sample size. Considering the high variability on some of the cognitive tasks used in this study it is difficult to discern the stability of the results – positive or negative - presented here. This is particularly true for the BRCA control group, which only contains 4 people. Low sample size also influences correlation results as outliers may skew results. For example, five of the eight women who have undergone BSO are approximately 1-year post surgery. More women 2 to 7 years post surgery are needed to establish the validity of observed correlations between time since oophorectomy and performance on cognitive tasks. Low sample size coupled with variability within groups underscores the necessity for larger samples. As discussed above, variability in the two control groups is higher than that in the BSO group on a number of tasks. To compensate, where appropriate, non-parametric tests (Kruskal-Wallis and Mann Whitney) were used in place of ANOVAs and post hoc LSD comparisons.
Another limitation is that simply receiving a diagnosis of a BRCA mutation is an extremely difficult life event; the stress of which many indirectly affect cognition. We have tried to control for this by administering the CES-D and excluding the results of anyone who obtains a score indicating they are depressed. There is also the possibility that the BRCA genes plays a role in cognition and that a BRCA mutation might adversely affect memory. Though there is no research to suggest that a BRCA mutation would directly affect cognition, this possibility cannot be ignored. The BRCA control group is intended to serve as a control for the role of BRCA mutation in directly and indirectly altering cognition. However, given the low number of participants in that group currently, the BRCA control group cannot sufficiently exclude these factors.

Another limitation is that three women in the BSO group were taking ERT at the time of testing. Ideally, we would like to exclude women on ERT, but an a priori decision was made to keep them in all analyses until the sample size is larger. As opinions on the impact of ERT use on cancer risk following oophorectomy are divided (Rebbeck et al., 2002), the healthy user bias may play a role in which women select to use ERT. The healthy user bias argues that overall, healthier and better-educated women are more likely to use HRT (Sherwin, 2005). However, as most of the women in the BSO group are well educated, particularly on areas concerning their health, it is unlikely that this is the case. Additionally, due to time constraints on data collection, we were not able to test all control participants during the early follicular stage of the menstrual cycle (menses), when ovarian hormones are lowest. As hormones fluctuate throughout the month, this may contribute to some of the variability in scores seen in the two control groups.

A further limitation of the present study is that study design and recruitment prevented the study from being blinded. The experimenter was aware of group membership at the time of testing. Though conscious effort was made to ensure consistency in participant testing across all participants, tester action can affect test results (Day & Altman, 2000).

13.5 Conclusion

In summary, we hypothesized that BSO would lead to changes in hippocampal and frontal cortical functioning. Our findings suggest that surgical menopause has a negative effect on verbal memory and attention but a positive effect on spatial memory and fluency clustering.
This indicates that removal of the ovaries critically alters cognitive functioning and underscores a relationship between ovarian hormones and women’s brain health.
References


double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. *Menopause, 13*(3), 411-422.


# Appendix A: Individual Scores

Table 16: Individual Scores – Demographics and Hormone Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Years)</th>
<th>IQ</th>
<th>E1G(ng/mL)</th>
<th>PdG (µg/mL)</th>
<th>Time since BSO (Years)</th>
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<tr>
<td>1 BSO</td>
<td>48</td>
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Table 17: Individual Scores – Logical Memory and Fluency

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Table 18: Individual Scores – Rey Auditory Verbal Learning Test
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Table 19: Individual Scores – R/K task and Object Placement Task

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<th>R/K Hits</th>
<th>R/K Proportion Remembered</th>
<th>R/K Proportion Familiar</th>
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<th>OPT Immediate Displacement</th>
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Table 20: Individual Scores – SPWM, DOT, Digit Span and Corsi Block Tapping Task

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<th>DOT Total</th>
<th>DOT Average</th>
<th>Digit Span Forward</th>
<th>Digit Span Backward</th>
<th>Corsi Block Span</th>
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</table>
Appendix B: Analyses rerun excluding women on ERT

**Logical Memory**

Excluding women taking ERT there was no significant effect of group on Delayed recall $F(2,13)=0.911, p=0.426$. The preplanned contrast between the BSO and Control groups was not significant $t(13)=-1.142, p=0.274$. Correlations between Time since oophorectomy and Immediate and Delayed recall were still not significant $r(3)=0.215, p=0.728; r(3)=-0.205, p=0.740$.

**Semantic and Phonemic Fluency**

Excluding women taking ERT there was no significant effect of group on Semantic switching $F(2,13)=0.178, p=0.839$. Preplanned contrasts revealed no differences were found between BSO and Control groups $t(13)=0.351, p=0.731$. Further, there remained a marginally significant effect of group on semantic cluster size $H(2)=5.169, p=0.069$ and a marginally significant difference between cluster sizes in the BSO and Control groups $U=12,500, p=0.090$.

Excluding women taking ERT there was no significant effect of group on phonemic fluency total $F(2,13)=0.953, p=0.411$; phonemic cluster size $F(2,13)=2.200, p=0.150$; or phonemic switching $F(2,13)=0.206, p=0.816$. Preplanned contrasts comparing BSO and Control groups were not significant for phonemic fluency total $t(13)=1.106, p=0.328$ or phonemic switching $t(13)=-0.273, p=0.789$. Correlations between Time since oophorectomy and semantic fluency total, cluster size were not significant $r(3)=-0.654, p=0.232; r(3)=-0.243, p=0.693, r(3)=-0.327, p=0.591$. Correlations between Time since oophorectomy and phonemic cluster size and switching were also not significant $r(3)=-0.463, p=0.432; r(3)=-0.625, p=0.259$.

**Rey Auditory Verbal Learning Test**

Excluding women taking ERT, there was no significant effect of group on items recalled in Trials A1-5, Trial B and Trial A6. There was no significant effect of group on Retroactive interference $F(2,13)=2.479, p=0.122$, Proactive interference $F(2,13)=1.539, p=0.251$ or Recency Score $F(2,13)=0.041, p=0.960$. 
Object Placement Task

Excluding women on ERT there was no significant effect of group on Immediate or Delayed object displacement $F(2,14)=0.561, p=0.583; F(2,14)=0.334, p=0.721$. Preplanned contrasts between the BSO and Control groups were still not significant. When Years of educations and BSO status were entered into a regression model with Immediate object displacement as the dependant variable, the overall model remained significant $F(2,14)=4.488, p=0.031$. Years of education and BSO status remained significant predictors $p=0.017, p=0.050$. The correlation between Time since oophorectomy and Immediate object displacement remained not significant $r(3)=-0.695, p=0.192$.

Remember Know

Excluding women taking ERT there was no significant effect of group on Items correct $F(2,10)=0.016, p=0.984$; Hits $F(2,10)=0.301, p=0.747$; Proportion remembered $F(2,10)=0.716, p=0.512$; Proportion familiar $F(2,10)=0.561, p=0.588$ and False alarms $F(2,10)=0.040, p=0.961$. Preplanned contrasts revealed no significant differences between the BSO and Control groups or between Age and BRCA control groups on any of the variables.

Spatial Working Memory Task

Excluding women on ERT there was no significant effect of group on Trial 1 errors $F(2,14)=0.326, p=0.727$; Trial 2 errors $F(2,14)=0.203, p=0.819$; Total errors $F(2,14)=0.412, p=0.670$ or Total time $F(2,14)=0.363, p=0.702$. Preplanned contrast between women with BSO and Controls were still not significant. The correlations between Time since oophorectomy and Trial 1 errors, Trial 2 errors, Total errors and Total time were not significant.

Digit Ordering Task

Excluding women on ERT there was no significant effect of group on Total Errors $F(2,13)=0.555, p=0.587$ or Average Time $F(2,13)=0.720, p=0.505$. In addition there were no significant differences between women with BSO and Controls on Total Errors or Average Time.
Correlations between Time since oophorectomy and Total Errors $r(3)=0.470$, $p=0.425$ and Average Time $r(3)=-0.631$, $p=0.254$ were not significant.

**Digit Span and Corsi Block Tapping Task**

Excluding women on ERT there was no significant effect of group on Digit Span Forward $F(2,13)=0.485$, $p=0.627$; Backward $F(2,13)=0.169$, $p=0.846$ and Corsi Block Span $F(2,14)=0.141$, $p=.870$. Preplanned contrasts between women with BSO and Controls were not significant. Correlations between E1G or PdG concentration and Digit Span Forward, Backward and Corsi Block Span were not significant.