The Effect of 17β-estradiol on Cognition

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Abstract

A bilateral salpingo oophorectomy (BSO), removal of the ovaries, is recommended for carriers of BRCA1/2 mutations to reduce cancer risks. However, BSO induces surgical menopause, and is associated with an increased risk of dementia (Rocca et al., 2007a) and Parkinsonism (Rocca et al, 2007b). This study investigated the cognitive outcomes of BSO one to ten years postsurgically. The present dataset (n=37) revealed there was a significant difference between the BSO group and their age matched cohort on the Logical Memory task assessing verbal, episodic memory. Levels of E1G (estrogen metabolite) was a significant predictor of the RAVLT primacy subscale indicating higher levels were associated with better recall at the beginning of a list of words. Controlling for age, performance on the RAVLT A1 measuring short-term memory degraded further out from BSO. While limited by the sample size, results are consistent with reports of post-BSO cognitive changes (Vearncomb & Panchana, 2009).
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Chapter 1

1 Literature Review

1.1 Overview
It has long been understood that sex steroids cause differentiation of secondary sexual characteristics, but how they affect different domains of cognition is less apparent. To isolate the effects of estrogen on cognitive function, the present study is based on women with the BRCA1 or BRCA2 mutation (BRCAm) who have undergone a prophylactic bilateral salpingo oophorectomy (BSO), the surgical removal of their ovaries and fallopian tubes. In absence of E2 produced by the ovaries, this population provides the unique opportunity to identify which cognitive functions are affected and to observe the trajectory of changes over time.

1.2 BRCA mutations and BSO
The BRCA gene is a tumour suppressor gene implicated in DNA repair, and deleterious mutations of this gene are associated with increased risks of breast and ovarian cancers. Development of ovarian cancer for women up to 75 years old with intact ovaries is estimated to be 62% in BRCA1 and 18% in BRCA2 carriers (Finch et al., 2006). Upon detection, carriers of the mutation are advised to undergo rigorous, periodic testing of breast and ovarian cancer. A carrier at high risk due to a family history, for instance, may be counselled to undergo BSO as prophylaxis. This procedure is recommended after childbearing (Happe, 2006), but prior to the age of 40 for optimum protection (Eisen et al., 2005). In receiving a BSO, the risk of breast cancer is reduced by approximately 50% (Eisen et al, 2005), and that of ovarian, fallopian and peritoneal (membrane of the abdominal cavity) cancers by 80% (Finch et al., 2006).

Despite reducing the risk of hereditary gynaecological cancers, an oophorectomy below the age of 45 is associated with increased overall risk of mortality (Rocca, Gossardt & de Andrade, 2006), and before menopause: Parkinsonism, Parkinson’s Disease (Rocca et al., 2007a) and dementia (Rocca et al., 2007b). Similarly, the removal of the uterus, a hysterectomy, incurs an elevated risk of ovarian failure despite conservations of the ovaries. Independent of indication for
hysterectomy, the mean age of menopause of hysterectomized women (45.4 ± 4.0) occurs significantly earlier than controls (49.5 ± 4.04) with intact uteruses (Siddle, Sarrel & Whitehead, 1967). One line of evidence suggests a hysterectomy leads to early ovarian failure due to the altered hemodynamics of the ovaries (Ahn et al., 2002).

Endocrine changes following BSO are not equivocal to that of a natural menopause. Because the ovaries are a major source of E2, the supply of endogenous estrogen abruptly ceases, inducing a surgical menopause. At menopause, steroidogenesis continues in the ovaries at a reduced capacity (Meldrum, 1981); testosterone is produced in the greatest quantities, followed by androstenedone, estrone, and dehydroepiandrosterone (Ushiroyama & Shigimoto, 1995). In naturally menopausal women, the peripheral aromatization of androstenedione from the ovaries and adrenal glands to estrone accounts for most of the circulating estrogens (Grodin, Shteri & MacDonald, 1973). However, there is a significant reduction of bioavailable testosterone post-oophorectomy, which can be aromatized into 17β-estradiol (Laughlin et al., 2000). Effectively, a BSO causes deprivation of E2 and its precursor.

There is no doubt that carriers of BRCA1/2m who are at high risk of breast and ovarian cancers that undergo a prophylactic oophorectomy benefit from a reduction of risk in these cancers, but the procedure elicits a surgical menopause. This is a different endocrine state from women in natural menopause, and may cause them to be prematurely deprived of the estrogens produced by the ovaries as early as twenty years before natural menopause if a woman elects the surgery in her thirties. Premature estrogen deprivation has systemic consequences (Shuster et al., 2008), and the changes in cognition follow oophorectomy form the premise of this investigation.

1.3 Estrogen and the Neurobiology of Cognition

Estrogen receptors, classified into ER-α and ER-β, belong to the family of G-coupled receptors. ER-α is expressed in the ovarian stroma cells, abundantly in the uterus (Press et al., 1984) as well as bone cells (Eriksen et al., 1988), whereas ER-β has been found in the thymus, spleen, ovaries, testes, skin, lung, kidney and pituitary gland (Mosselman, Polman & Dijkema, 1996; Brandenberger et al., 1997). That ER-α and ER-β are localized in the hippocampus (Gonzalez et al., 2007), the frontal cortex (Bixo et al., 1995), hypothalamus, basal ganglia and amygdala
(Donahue, 2000), evidences the biological plausibility that estrogens may subserve cognition.

More direct support of estrogen’s role in intracellular signaling and synaptic plasticity comes from work in animal models. The acute application of estrogen, both in vivo and in vitro, has an excitatory effect on various brain regions and alters the resting membrane potential (Woolley, 2007). Further, estrogen has been demonstrated to promote the formation of dendritic spines in the CA1 neurons of the rodent hippocampus (Woolley & McEwen, 1992) as well as excitatory synapses (Woolley & McEwen, 1993). The role of E2 in long-term potentiation has also been studied in hippocampal slices. Specifically, high frequency administration of 100 pM of E2 induced greater long-term potentiation in the CA1 than the control (Foy et al., 1999).

Spatial working memory is a domain of cognition where the effects of ovariectomy (OVX) have been extensively studied. The radial-arm maze is a task commonly used in rats to assess spatial memory. In this paradigm, rats are placed on a platform with eight arms extending distally and trained to visit each arm, typically to obtain a food reward. Errors are counted as visits on arms where the reward has already been claimed as well as any missed arms. Using the radial-arm maze, Bimonte and Denenberg (1999) found that OVX was associated with a performance decrement to young rats. In the seven trials conducted, OVX rats made significantly more errors on the second to fifth trials. Further, OVX rats treated with subcutaneous capsules of physiologically low or moderate levels of E2 outperformed those that received the placebo for working memory demands of up to four items.

A more radical approach of delineating the role of estrogens in cognition has come from the knockout of the ER-β. Rissman et al. (2002) compared how OVX in mice between 5 and 7 months with a knockout of the ER-β (ERβKO) would affect performance of mice on a spatial memory task, and reported ER-β was necessary for optimal learning. After OVX, mice were treated with subcutaneous E2 (a high or low dose), or a placebo and tested ten days later. Over the four days of testing on the Morris water maze, it was observed that ERβKO mice, despite receiving E2, experienced delayed spatial learning acquisition relative to the wildtype. Another study using both male and female ERβKO mice indicated the knockouts showed severe memory impairment in a fear conditioning paradigm, and deficits in the hippocampal CA1 synapses in both sexes (Day et al., 2005).
In humans, a multitude of estrogenic effects in the brain has been documented in the literature. A decline of E2 following menopause has been associated with neurodegenerative diseases (see [Brann et al., 2007] for a review). For instance, women have a reduced risk of stroke until menopause (Roquer, Campello & Gomis, 2003), after which they suffer more aversive outcomes than men (Niewada et al., 2005). The increased incidence of Alzheimer’s disease and Parkinsonism reported in women after surgical menopause also suggest that the presence of E2 protects against neuropathology (Rocca et al., 2007a; 2007b).

Clearly, estradiol is implicated in a number of functions, and its decline associated with neuropathologies and cognitive impairment. Animals with OVX or an ER-B knockout show the disruption of estrogen production or functionality interferes with spatial working memory and learning. In this perspective, it is conceivable that surgical menopause in a young women may be cognitively detrimental. In the next section, a sampling of the literature on cognitive changes post-oophorectomy will be presented, as well as the effects of estrogen replacement therapy (ERT) use peri- or post-menopause.

1.4 Cognitive Outcomes of an Oophorectomy & Hormone Replacement Therapy

An oophorectomy performed before menopause is associated with numerous consequences: increased risk of overall mortality (Rocca et al., 2006), immunodeficiencies (Kumru, Godekmerdan, & Yilmaz, 2004), sleep disturbances (Benshushan et al., 2009), cardiovascular disease (Atsma et al., 2006), osteoporotic fracture (Melton et al., 2003), depression (McKinlay, McKinlay & Brambilla, 1987), Parkinsonism (Rocca, Grossardt & Maragnaore, 2008), and of particular interest, memory decline (Shuster et al., 2008). Despite that women with BRCA mutations are a major constituent for whom oophorectomies are indicated, there are no studies to date investigating cognitive changes specific to this population post-surgically.

The Mayo Clinic Cohort Study of Oophorectomy and Aging generated provocative results suggestive that estrogen deprivation may lead to an increased risk of Parkinson's Disease (PD) and Parkinsonism following surgical menopause (Rocca et al, 2007a). A cohort of 2327 women compared to 2368 referent women were followed by a research team through telephone interviews and an examined by a movement disorders specialist for those who screened positive for PD. A diagnosis of PD was made based on medical records, death certificate documentation
of PD, a previous diagnosis reported by women who possessed inadequate medical records or could not be examined. If a participant was incapacitated due to deafness, cognitive impairment or terminal illness, a proxy informant was interviewed instead. There was a significantly increased risk of Parkinsonism in women who had undergone BSO compared to controls, adjusted for years of (HR = 1.80) and an increased. The risk for developing PD was increased, but non-significant ($p = 0.15$). A younger age of BSO was significantly correlated with a higher risk. Likewise, a unilateral oophorectomy was associated with a significantly increased risk of Parkinsonism as well, and the risk was also higher in women who had underwent the surgery at a younger age.

In a subset of the Mayo Clinic cohort, it was found that the risk of cognitive impairment for developing dementia was higher in women with oophorectomies (Rocca et al., 2007b). A total of 1489 women who had undergone unilateral or bilateral oophorectomies before natural menopause for non-cancer related indications were administered the 12-item Telephone Interview for Cognitive Status-modified (TICS-m). Again, a proxy informant was interviewed if the participant was incapacitated, using the Brief Dementia Questionnaire. Women were considered to be cognitively impaired if they scored greater than 27 out of 30 on the TICS-m, if the proxy reported a previous diagnosis of dementia, senility or AD, or impairment of daily activities due to cognitive problems. Compared to the referent cohort, a 1.70 hazard ratio for developing AD was found for women who had undergone a BSO under the age of 49. In addition, there was a greater risk of AD with younger age of surgery.

Sherwin (1988) conducted the first RCT investigating changes in memory on 43 participants who had undergone a BSO. Participants were randomly assigned to an estrogen (estradiol valerate) group, androgen (testosterone enanthate) group, estrogen-androgen group (testosterone enanthate benzilic acid hydrozone, estradiol dienanthate and estradiol benzoate), or placebo group and given a monthly intramuscular injection. A hysterectomy group was also tested at baseline, three, four, and seven months after surgery without intervention as control for a urogenital surgery. The Digit Span test, Clerical Speed and Accuracy, Paragraph Recall, and Abstract Reasoning test were administered to all participants at four time points in a counterbalanced order. By seven months post-surgery, the placebo group performed significantly worse on all four tasks, coinciding with their lower levels plasma concentrations of estradiol and
testosterone. The hysterectomy controls remained stable in concentrations of ovarian steroids and performance over time, indicating a null effect of a surgery. Although the sample size was small, these were the first results that directly evidenced the importance of sex steroids in the maintenance of memory.

Three studies conducted since Sherwin's (1988) seminal findings have compared cognitive functions of women pre and post-surgery, all of which reported a decline in post-surgically. Sherwin and Phillips (1990) assessed cognitive function in 12 women with hysterectomy and BSO in a randomized placebo-controlled trial, and found better performance in verbal memory in the E-treated group two months post-surgery. With a slightly larger sample, Phillips and Sherwin (1992) then tested 19 women two weeks pre-operatively, and again two months after treatment with three monthly injections of 10mg E2 valerate or sesame oil. Those receiving the active injections performed significantly better on the LM task than the placebo group. Furthermore, on both the immediate and delayed conditions of the Paired Associates test, the number of words correctly recalled by the placebo group was significantly reduced between the pre and post-operative tests. Lastly, Farrag (2002) showed that 35 women with hysterectomy and BSO performed worse than controls on the MMSE, as well as the Digit Span, Associate Learning, and Logical Memory tests at six months postoperatively.

Evidence in favour of the neuroprotective effects of estrogen was largely unchallenged until results of the Women’s Health Initiative Memory Study (WHIMS; Espeland et al, 2004; Coker et al., 2010) were published. The WHIMS was a large scale, longitudinal randomized control trial, designed to address how conjugated equine estrogen (CEE) replacement therapy affected cognitive functioning in postmenopausal women. Between 1982 and 1992 CEE with medroxyprogesterone acetate (MPA) was the most common preparation for hormone therapy prescribed to menopausal women in the United States (Wysowski, Golden & Burke, 1995). Randomized trials were conducted with CEE alone and CEE with MPA, but prematurely terminated due to the unforeseen increased risk of dementia and cognitive decline. A 2.05 hazard ratio of probable dementia and decrease in general cognitive function as assessed with the MMSE was reported for participants assigned to the CEE + MPA trial compared to the placebo. Participants assigned to the CEE trial alone also experienced a 49% higher incidence of developing probable dementia (37 cases, instead of 25 in 10000), although the differences
between both hormone preparation groups and placebo groups were not significant ($p = 0.18$).

A subset of participants from the WHIMS were enrolled in the ancillary Women’s Health Initiative Study of Cognitive Aging (WHISCA), also a randomized control trial, to address the effects of ERT on memory, working memory, attention, spatial abilities, motor performance, mental speed and executive function (Resnick et al., 2004). CEE alone was associated with poorer spatial rotation abilities, and in conjunction with MPA, lower verbal ability. For postmenopausal women over 65-years old, the WHIMS and WHISCA provided compelling evidence that hormone preparations of CEE with or without MPA were associated with negative cognitive outcomes.

A proposal by Resnick and Henderson (2002) that estrogenic effects on cognition are time-sensitive may reconcile the inconsistent findings in the literature. The critical period hypothesis postulates that ERT only endows a benefit when started perimenopause (Rocca, Gossardt & Shuster 2010). Women who received a BSO and were treated with estrogen through the age of 50 exhibited the same risk of dementia and cognitive impairment as the general population (Rocca et al., 2007a), suggesting that the use of ERT mitigated any additional risk of dementia. On the other hand, the WHIMS illustrated treatment with CEE and CEE with MPA can have a detrimental effect if used well after menopause. Participants of the WHIMS averaged 73 years old at time of recruitment, compared to the mean age of menopause at 51 (Kato et al., 1998).

Despite the mixed findings in the literature, results can be reconsolidated into a framework that fits more consistently with the data if time of oophorectomy and age of ERT initiation are taken into consideration. A number of studies have reproduced the effect that BSO is detrimental to verbal episodic memory (Henderson & Sherwin, 2007). At the same time, that surgical menopause is not associated with significant changes to cognitive functions have also been reported (e.g. Kritz-Silverstein & Barrett-Conner, 2002). A holistic interpretation of the literature is problematic because of methodological heterogeneity across studies. Nevertheless, focusing on studies of pre- and post-BSO performance leads us to believe there are cognitive changes after surgical menopause.
1.5 Apolipoprotein E

In addition to E2 deprivation as a predictor of cognitive decline, we believe that apolipoprotein E (APOE) genotype will also have bearing on individual outcomes. Convergent evidence has suggested that the ε4 variant of the APOE gene is associated with an elevated risk of developing Alzheimer’s disease (Corder et al., 1994; Hofman et al., 1997), and for individuals with mild cognitive impairment, the transitional state between normality and dementia (Small, Rosnik, Fratiglioni, & Backman, 2004). The apoE protein comprises a family of lipid transport proteins that also have a role in neuronal repair and outgrowth (Mahley & Rall, 2000). The ε4 heterozygotes have a risk between 3 to 4 times greater of developing Alzheimer’s disease, compared to a 10 to 12 times increased risk in ε4 homozygotes (Farrer et al., 1997), indicating that gene dosage affects cognitive outcome.

A meta-analysis has indicated that the ε4 allele is associated with impairments in global cognitive functioning, episodic memory and executive functioning, despite effect sizes being relatively modest (Small et al., 2004). A total of 38 studies published between 1993 and 2004 based on non-demented individuals were used in this review, and neuropsychological tests categorized into testing one of eight cognitive domains. Effect sizes calculated using Cohen’s d ranged from -0.03 to -0.06, with the largest found with tests assessing executive function (e.g. Trailmaking test, or the Wisconsin Card Sorting Task). Comparing ε4 carriers to E3 homozygotes, an effect of zygosity was found, such that ε4 homozygotes performed significantly worse on tests of global cognitive functioning. Despite a fourfold risk of E4 carriers developing AD, cognitive defects in older adults were at best, 9% of a standard deviation in magnitude – suggesting any effects of APOE we observe would be likewise be small.

Recently, it has been theorized that APOE represents a form of antagonistic pleiotropy, characterized by its differential effects throughout the lifespan (Tuminello & Han, 2011). According to this view, children with the ε4 allele should outperform non- ε4 carriers on cognitive tests, and it is not until later life that the ε4 is detrimental. Support in favour of this theory is mixed. For instance, there is evidence of apoE4 carriers (11 to 16 years old) having better visuospatial ability (Bloss et al., 1999), children (5 to 7 years old) with apoε4 showing dysfunction on the Differential Ability Scales (Gozal et al., 2007). The consensus is that apoE4 is not associated with an appreciable difference in cognitive functions until approximately age 50.
and onwards (Tuminello & Han, 2011). Corroborating this theory, Small et al. (2004) also reported a negative correlation between age and magnitude of ε4-associated cognitive deficits, but this effect did not reach statistical significance.

Animal studies further suggest there may be an interaction between estrogen and APOE. One line of research has investigated the regulation of apoE by E2 in the glia in rats (Stone et al., 1997), building on previous work indicating that levels of estrogens in the blood is associated with decreased plasma apoE (Muesing et al., 1992). Nine-month old female rats were sacrificed at diestrus, proestrus or estrus, and brain tissue examined by immunohistochemistry. The levels of apoE mRNA were found to be significantly higher in the hippocampal CA1 region and hypothalamic arcuate nucleus during proestrus, when estrogen levels peaked. *In vivo*, Stone et al. (1997) reported that E2 administration induced a significant increase in apoE mRNA in a mixed glia culture.

There is evidence indicating the ε4 allele has detrimental effects on cognitive outcomes, and is associated with dementia. That the ε4 allele can interact with E2 can also be found in the animal literature. As well, using the extant literature on women aged 50-years-old and up, it can be extrapolated that ε4 carriers will experience decline in certain cognitive domains compared to non-carriers. By observing cognitive performance of ε4 carriers in oophorectomized women, it will be possible to determine how estrogen interacts with the APOE gene.

Thus in this study, we studying cognitive changes following oophorectomy out to ten years post-surgically. There is evidence suggesting E2 is important to the maintenance of a number of bodily systems and potentially their interactions (Einstein et al., 2012). It is an important nuance to make that this study does not resolve to determine how low levels of estrogen is related to cognitive functions *per se* (i.e. in menopause), but the consequences of an early deprivation. Because it is recommended that a BSO is performed prior to 40-years-old (Eisen et al., 2005), a woman may experience surgical menopause approximately ten years before the average age of natural menopause.
Chapter 2

2 Methods

2.1 Participants

Three groups were included in the study: women who were carriers of the BRCA1 and/or BRCA2 mutation (BRCAm), carriers who had undergone a bilateral salpingo-oophorectomy before natural menopause (BSO), and age-matched controls (AMC), all of whom were between the ages of 30 to 50 years old and fluent English speakers. An AMC group was included because an appreciable age difference was expected between the BRCA and BSO group, as childbearing factored into the decision to undergo the surgery. The exclusion criteria for all groups were pregnancy, depression, the ongoing use of tamoxifen, or aromatase inhibitors. The exhaustive exclusion criteria was devised to eliminate any confounds due to exogenous hormones or circumstances that could alter cognitive performance.

BRCA carriers and the BSO group were recruited from the Familial Breast Cancer Research Unit at Women’s College Research Institute (WCRI) led by Dr. Steven Narod. Potential recruits were sent an introductory letter outlining what this study entailed with an invitation to participate, and followed up with a telephone call approximately two weeks after the letters were posted. Participants who had previously been tested received a recall letter acknowledging our appreciation of their continual support of the study, and invited to continue participation. Lastly, age-matched controls were recruited through a notice posted on the University of Toronto’s online bulletin, as well as advertorial posters around campus.

2.2 Neuropsychological battery

An array of 12 psychometric tasks was selected to assess memory, working memory and attention. These tasks were selected based on their sensitivity, and included a mixture of tasks used in the literature clinically to study AD or MCI, as well as in basic research. An additional self-report questionnaire was used to assess mood. Individuals were asked to give informed consent of their participation after reading an information sheet describing the purpose of the study, and their rights as a participant. Next, participants filled out a demographic package partitioned in five sections: background information, reproductive history, BRCA mutation and
prophylactic surgery, breast cancer and other cancers, and medications.

Tasks ranged from 3 to 15 minutes in duration, administered by an experimenter in a private, soundproof room. The entire appointment typically lasted 90 minutes. There were no risks to this study, although the repetitive nature and difficulty of some tasks may have produced minor fatigue, boredom or mental strain. As such, participants were advised to take a break any time in between tasks if needed. More challenging tasks (e.g. the Mental Rotation Task or Digit Ordering Task) were interspersed with less demanding ones (e.g. North American Adult Reading Test) but the battery was overall designed to be difficult to prevent ceiling effects. Tasks were administered in the order below once participants indicated they were ready to begin.

1. **Verbal Fluency** (Strauss, Sherman & Spreen, 2006): In the phonemic fluency component (also known as the Controlled Oral Word Association Task; Benton et al., 1994), participants were given a minute to verbally produce as many words that began with a certain letter, exempting proper nouns and variations of a word. For instance, given the letter “T”, “Thomas”, and “tobogganing” said after “toboggan” were inadmissible. Three letters were given at each time point: in F, A, S in year one and three, and C, F, L in year two. In the semantic fluency component (Strauss, Sherman & Spreen, 2006), participants were once again given one minute to produce as many animals (year one and three) or items found in a supermarket (year two) as possible. The number of words, mean cluster size, and number of switches were scored (see Troyer, Moscovitch & Winocur, 1997) for each letter or category.

2. **Logical Memory** (LM; Wechsler, 1945): the LM comprises short narratives consisting of approximately three sentences that were read aloud to the participant, after which they were asked to immediately recall as many details from the story as possible. Approximately twenty minutes later, participants were once again asked to recall everything they remembered from each story. Points were scored for recalling details of the narrative verbatim, and also for conveying the ideas synonymously. The Logical Memory test is one of verbal, episodic memory.

4. **Spatial Working Memory Task** (SPWM; Duff & Hampson, 2001): the SPWM consists of ten pairs of coloured dots hidden beneath a board with twenty hinged doors. Participants were
instructed to simultaneously lift up two pairs of doors in search of matching colours in as few attempts as possible. The task was completed once all ten pairs of colours were located, and a second trial commenced thereafter. Time taken to complete the task and the coordinates of doors opened on each trial was recorded, which was used to determine the number of working memory errors. The SPWM is a rigorous test of spatial working memory, and in turn, function of the prefrontal cortex (Duff & Hampson, 2001). Performance has been shown to correlate significantly with levels of estrogen in women (Duff & Hampson, 2000; 2001).

5. Digit Span Task (DS; Wechsler, 1945): in the DS forward, the experimenter read a sequence of digits, which the participant was instructed to repeat. There were two trials per sequence length, and the length of each sequence increased if participants correctly reported the entire span. If the first sequence of a pair at n-length was incorrect but the second was correct, the experimenter continued to the subsequent sequence that was n+1 digits long. The forward component is a test of verbal memory. In the backward component of the DS assessing working memory, the experimenter read a sequence and participants were instructed to repeat this sequence in the reverse order. Once again, as long as one of the two sequences was correctly reported, the length of the digit span increased.

6. Logical Memory (Delayed): Approximately 15 minutes after hearing the two narratives, participants are asked to recall as many details as possible a second time.

7. Object Placement Task (OPT; Smith & Milner, 1981; Crane & Milner, 2005): in the OPT, participants were instructed to name, and study images of everyday objects (e.g. a tree, tire, water bottle) arranged on a 60 cm square board for one minute. Next, participants were asked to replicate the spatial arrangement of the images by pinning them on a blank corkboard. The X and Y coordinates of each object were measured at the end of the test session to obtain displacement from their true positions and in effect, spatial memory.

8. Rey Auditory Verbal Learning Task (RAVLT; Lezak, 1983): the RAVLT is a verbal learning and memory. Participants were instructed to repeat as many words recalled from a list of fifteen nouns read to them by the experimenter. Four additional trials of reading by the experimenter and recall by the participant were repeated, and a new list of fifteen words was
presented orally after. Participants were asked to reproduce the new list of words, and for a final time, the first list of words.

9. **Object Placement Task** (delayed): approximately 15 minutes after viewing of the OPT board, a second trial was administered.

10. **Corsi Block Task** (CBT; Lezak, 1995): the CBT was performed on a board affixed with ten numbered cubes that faced the experimenter. Participants were instructed to replicate the sequence the experimenter tapped on the top face of the cubes. Again, there were two trials per sequence length, and the participant advanced to the next sequence if she correctly tapped the cubes in the order presented. The CBT tests passive, visuospatial short-term memory (Kessels, 2000).

11. **Digit Ordering Task** (DOT; Petrides et al., 1993): the DOT comprised ten trials wherein participants were asked to generate the numbers from one to ten inclusive in a random sequence. The use of patterns was not permitted. Spontaneous use of the fingers as visual aid by the participant was allowed, but not encouraged. In the ten trials conducted, the number of repetition (i.e. repeated digits) and omission (i.e. any digit from 1 to 10 that was left out) errors, and the time to produce each set was recorded. The DOT is a verbal working memory task, and performance has been linked to the mid-dorsolateral prefrontal cortex (Petrides et al., 1993).

12. **North American Adult Reading Test** (NAART; Blair & Spreen, 1989): the NAART comprised a list of 61 irregularly pronounced words which participants were instructed to read aloud, and used to quickly assess intelligence in lieu of a time consuming, full scale IQ test. The NAART was only administered once for each participant upon intake.

13. **Mental Rotation Task** (MRT; Vandberg & Kuse, 1978; Peters, 2005): the MRT consisted of fifteen questions depicting a three-dimensional reference figure, and four additional test figures. Participants were instructed to determine which two of the test figures corresponded to the reference figure depicted in a different orientation. In order to score two points per question, both correct answers had to be marked; questions with only one of the two correct
figures selected did not receive a partial score. The MRT is another spatial task, but unlike the CBT, it requires the active manipulation of objects.

14. Centre for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977): the CESD is a 20-item self-report questionnaire used to assess mood on a four-point Likert scale. Participants were asked to indicate the frequency of which a statement during the past week – for example, “my sleep was restless”. A total score of 16 or greater is suggestive of depressive symptomology. Any individual scoring above this cut-off was not included in the data analysis and referred to a social worker if needed.

2.3 Hormone and genetic testing
Upon completion of the test session, a urine and saliva sample was collected. Participants were asked to provide a 2mL saliva sample collected in an OraGene DNA collection kit (OGR-500) and then directed to the bathroom to provide a small urine sample in a sterile cup. Participants were thanked for their time, given a $10 gift card to Second Cup and reimbursement for transportation fees. Urine samples were stored in a freezer and the saliva in the Oragene DNA kits at room temperature until samples were delivered to the Centre for Biological Timing and Cognition at the University of Toronto, and Women’s College Hospital respectively for analysis.

The level of estrone glucuronide (E1G) and pregnanediol glucorinide (PdG) was determined from the urine sample. Estrone glucuronide is a water-soluble metabolite of 17β-estradiol excreted from the kidneys via urine, and pregnanediol glucorinide is that of endogenous progesterone. The concentration of urine was determined by a competitive enzyme immunoassay carried out at the University of Toronto’s Centre for Biological Timing and Cognition, using polyclonal antibodies (E1G R522-2, PdG R13904) purchased from the University of California Davis.

The participant’s APOE genotype was determined from saliva samples collected in OraGene OG-500 kits. Analysis was conducted at the Women’s College Hospital by Dr. Steven Narod’s laboratory. Differences are found on the APOE alleles on codons 130 and 176; the ε3 allele has the amino acids cysteine and arginine respectively, ε4 has two cysteines, and ε4 with two
arginines. The region containing codons 130 and 176 were amplified, and the DNA fragment directly sequenced with the BigDye Terminator Cycle Sequencing kit using an ABI 3500xl DNA analyzer. Genotypes were determined based on the variations detected with SoftGenetics’ Mutation Surveyor software.

2.4 Statistical Analyses

Statistical analysis was conducted to determine: 1) whether group differences existed; 2) whether there is a relationship between the level of E1G in BSO participants and cognitive performance; 3) if performance changed as a function of time since BSO; 4) if carrying the E4 allele would have an impact on cognitive performance.

The majority of hypotheses were modeled after the effects found in the literature, based on cognitive changes post-BSO, or those in which improvements were associated with ERT after menopause (Table 1). The two hypotheses based specifically on the RAVLT A1 subscale were derived from prior data in this study. Although progesterone was measured from the urine, it served as an indication of the level of ovarian steroids present at the time of testing, and was excluded from analysis.

Table 1
Primary hypotheses of the study

<table>
<thead>
<tr>
<th>Task</th>
<th>Domain Tested</th>
<th>Hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM</td>
<td>Verbal, episodic memory</td>
<td>1. BRCA ≠ AMC ≠ BSO</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Non-E4 &gt; E4 carriers&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SPWMT</td>
<td>Spatial WM</td>
<td>3. Performance correlates positively with E1G</td>
</tr>
<tr>
<td>Digit Span</td>
<td>Verbal memory</td>
<td>4. Performance correlates positively with E1G</td>
</tr>
<tr>
<td>RAVLT A1</td>
<td>Verbal memory &amp; attention</td>
<td>5. Performance correlates negatively with time since BSO</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. Non-E4 &gt; E4 carriers&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>E4 carrier is used to describe an individual with at least one E4 allele

ANCOVAs were performed to analyze performance differences across the three experimental groups with CES-D scores used as a covariate. Levene's test of equality of variance was conducted to ensure assumptions of the ANCOVA were met. If the assumption of equal
variances was violated at \( p < .05 \), a non-parametric Kruskal-Wallis test was conducted instead. Any significant difference found at \( p < .05 \) with the ANCOVA, was further investigated with a pairwise comparison using a t-test to determine whether a significant difference existed between the hypothesized pairs of groups.

Multiple regression models were used to first test the contribution of estrogen to performance and time since BSO for each neuropsychological test, controlling for the effects of age, years of education, and CES-D scores. A forced entry method was used in all cases to enter predictor variables, and the models were screened for overall significance. Next, the contribution of the predictor variables of interest (E1G or time since BSO) to the model was checked for significance at \( p < .05 \).

To determine whether APOE genotype affected performance, a comparison was made between scores of the \( \varepsilon4 \) carriers (\( \varepsilon4 \) heterozygotes and homozygotes) and non-carriers (E2/E2, E2/E3, E3/E3). An independent t-test was used to determine if the difference between \( \varepsilon4 \) carriers and non-carriers was significant at \( p < .05 \).

Outliers were operationalized as two standard deviations within either direction of the mean when scores were normally distributed, and removed from that dataset prior to analysis. Q-Q plots were created for all measures to check for violations of normality before conducting parametric analyses. When a distribution was skewed or not normally distributed, a transformation was applied. In the present dataset, all measures with a non-normal distribution were transformed with a base 10 logarithmic function. Any participant in the BRCA or AMC group who reported to be menopausal at the time of testing was omitted from the data, as well as individuals scoring greater than 16 on the CES-D. Consequently, two participants each from the BSO and AMC groups were omitted due to their CES-D scores. As well, one participant each from the BRCA and AMC groups were omitted because they were menopausal at the time of testing.
Chapter 3

3 Results

In the interest of brevity, only significant results at a threshold of \( p < .05 \) (i.e. before correction for multiple comparisons), or marginally significant results are reported in full. Given these are preliminary data, the results at this stage should be viewed as a preview of emerging trends that may or may not persist due to the stochastic nature of the data. A discussion of the sample size needed to achieve significant results for a selection of neuropsychological tests is later presented based on extrapolation of current effect sizes (Appendix E).

3.1 Demographics

Data from 37 participants were included in the analysis. Of the 25 participants who indicated their ethnicity, 21 (84%) were of Caucasian descent (originated from any European country, the Middle East or north Africa), followed by 8% African American, 4% Hispanic and 4% South Asian. There were no significant differences in the BMI, years of education, or estimated IQ between experimental groups as determined with a one-way ANOVA. BMI ranged from 19 to 33 (mean = 24.47, SD = 4.17) and was not correlated to E1G levels \((r = .112, p = .516)\) (Table 2). The medical history of select participants with a prior diagnosis of cancer, or those who are currently using medications, is presented in Appendix A.

The mean ages of the BSO and AMC group were closely matched, but the BRCA group was approximately 6 years younger than either of the group groups (Table 2). A univariate ANOVA confirmed there was a significant difference in ages across the groups \((F(2, 34) =5.94, p <.01)\). Differences across the remaining demographic variables and CES-D scores were not significant.
Table 2

Characteristics of the sample population (n = 37)

<table>
<thead>
<tr>
<th></th>
<th>BSO (n = 10)</th>
<th>BRCA (n = 6)</th>
<th>AMC (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Age</td>
<td>44</td>
<td>4.7</td>
<td>37.67</td>
</tr>
<tr>
<td>IQ</td>
<td>109.3</td>
<td>6.9</td>
<td>113.0</td>
</tr>
<tr>
<td>Education (years)</td>
<td>16.6</td>
<td>1.6</td>
<td>17.0</td>
</tr>
<tr>
<td>BMI</td>
<td>24.5</td>
<td>4.1</td>
<td>24.4</td>
</tr>
<tr>
<td>E1G (ng/mL)</td>
<td>14.0</td>
<td>12.0</td>
<td>28.1</td>
</tr>
<tr>
<td>PdG (µg/mL)</td>
<td>0.54</td>
<td>0.60</td>
<td>1.58</td>
</tr>
<tr>
<td>CES-D Score</td>
<td>5.3</td>
<td>2.26</td>
<td>2.33</td>
</tr>
</tbody>
</table>

In the BSO group, E1G levels were not as low as might be expected as four participants were using ERT upon their intake (first year of testing). Three participants reported using Estradot, a transdermal patch delivering E2, and one participant reported using oral conjugated estrogens. Results excluding these participants can be found in Appendix B.

3.2 Frequency of APOE Genotypes

The prevalence of the E3 and E4 alleles in the sample was respectively 66% and 34% (63% and 37% excluding the omitted participants) (Table 3). These numbers fall into the range of APOE genotype frequencies in the North American population reported by Eisenberg, Kuzawa, and Geoffrey (2010). No E2 alleles were found in the sample.

Table 3

Number of participants with each APOE genotype by group

<table>
<thead>
<tr>
<th>Genotype</th>
<th>BSO</th>
<th>AMC</th>
<th>BRCA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3/E3</td>
<td>5 (6)</td>
<td>12 (13)</td>
<td>0</td>
<td>58.5% (59.3%)</td>
</tr>
<tr>
<td>E3/E4</td>
<td>3</td>
<td>3 (4)</td>
<td>4</td>
<td>34.4% (34.3%)</td>
</tr>
<tr>
<td>E4/E4</td>
<td>0 (1)</td>
<td>0</td>
<td>0</td>
<td>0% (3.1%)</td>
</tr>
</tbody>
</table>

Note. Genotype frequencies excluding individuals who were not in subsequent analyses due to their CES-D scores or menstrual status are indicated in the parentheses.
3.3 Test of Differences by Group

The Proactive Interference subscale was calculated as the difference between the number of words recalled on the A1 trial and A6 trial, with a score of 0 indicating the participant produced the same number of words on both trials, and a negative score indicating more words on the A6 than A1 (Vakil & Blanchstein, 1994).

A marginally significant main effect of group differences was found in the Proactive Interference subscale ($F(2,33) = 3.75, p = .054$), indicating a differential ability to retain memories of a list of words after the memory load is increased with a second list. A pre-planned, post-hoc comparison indicated the BSO group did not perform significantly worse than either the AMC group or the BRCA group (Table 4).

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>AMC</th>
<th>BRCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td><strong>Proactive Interference</strong></td>
<td>-4.80</td>
<td>1.62</td>
<td>-6.0</td>
</tr>
</tbody>
</table>

Although there were no group differences on the Logical Memory task, a pre-planned comparison revealed a significant difference between the BSO and AMC group on the total, immediate score ($t(27) = 2.30, p = .029$). On the delayed condition, there was once again a significant different between scores of the BSO and AMC participants ($t(25)= 2.20, p = .037$). BSO participants recalled significantly fewer details than the control group whether they were asked to recall details of the narrative immediately after it was read to them, or 15 minutes later (Table 1, Figure 1).
Table 5

Descriptive statistics of Logical Memory (n = 29)

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>AMC</th>
<th>BRCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Immediate</td>
<td>15.00</td>
<td>3.80</td>
<td>19.89</td>
</tr>
<tr>
<td>Delayed</td>
<td>12.20</td>
<td>3.49</td>
<td>16.12</td>
</tr>
</tbody>
</table>

Figure 1. There was a significant difference between the BSO and AMC group’s performance on the immediate recall of the Logical Memory task, an assessment of verbal, episodic memory. *p < .05

3.4 Effect of Estrogen Levels in Women with BSO

3.4.1 RAVLT Primacy

There was a significant, positive effect of E1G on the RAVLT Primacy scale, which reflects the number of times the first three words were recalled across the five RAVLT A trials ($B = 0.962$, $p < .01$). Results indicate higher levels of E1G were associated with the ability to retain words learnt in the beginning of a series (Table 6).
Table 6
Summary of regression analysis for variables predicting the RAVLT Primacy in women with BSO (n = 8)

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
<td>β</td>
<td>B</td>
<td>SE B</td>
<td>β</td>
</tr>
<tr>
<td>Education</td>
<td>.092</td>
<td>0.302</td>
<td>0.157</td>
<td>-.031</td>
<td>0.093</td>
<td>-0.054</td>
</tr>
<tr>
<td>Age</td>
<td>.047</td>
<td>0.125</td>
<td>0.203</td>
<td>-.019</td>
<td>0.039</td>
<td>-0.082</td>
</tr>
<tr>
<td>CES-D</td>
<td>.062</td>
<td>0.195</td>
<td>0.149</td>
<td>.051</td>
<td>0.059</td>
<td>0.124</td>
</tr>
<tr>
<td>E1G</td>
<td></td>
<td></td>
<td></td>
<td>.074</td>
<td>0.01</td>
<td>0.962</td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>.079</td>
<td></td>
<td></td>
<td>.932</td>
<td></td>
</tr>
<tr>
<td>F of R² Change</td>
<td>0.143</td>
<td></td>
<td></td>
<td>50.35**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**p < .01

3.4.2 RAVLT Recency
There was a significant, negative effect of E1G on the RAVLT Recency scale, which reflects the number of times the first last words were recalled across the five RAVLT A trials (B = -0.927, p = .003) (Table 7). Results indicate lower levels of E1G were associated with the ability to retain words learnt at the end of a series.

Table 7
Summary of regression analysis for variables predicting the RAVLT Recency (n = 8)

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
<td>β</td>
<td>B</td>
<td>SE B</td>
<td>β</td>
</tr>
<tr>
<td>Education</td>
<td>-.339</td>
<td>0.405</td>
<td>-0.433</td>
<td>-.040</td>
<td>0.052</td>
<td>-0.052</td>
</tr>
<tr>
<td>Age</td>
<td>-.032</td>
<td>0.122</td>
<td>-0.129</td>
<td>.037</td>
<td>0.148</td>
<td>0.148</td>
</tr>
<tr>
<td>CES-D</td>
<td>.057</td>
<td>0.300</td>
<td>0.091</td>
<td>-.081</td>
<td>0.100</td>
<td>-0.130</td>
</tr>
<tr>
<td>E1G</td>
<td></td>
<td></td>
<td></td>
<td>-.097</td>
<td>0.015</td>
<td>-0.947</td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>.135</td>
<td></td>
<td></td>
<td>-.927</td>
<td></td>
</tr>
<tr>
<td>F of R² Change</td>
<td>.260</td>
<td></td>
<td></td>
<td>12.72*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .05
3.5 Effect of Time since Oophorectomy on the RAVLT

The overall regression model using a forced entry method with age, years of education, CES-D scores showed that time since BSO significantly predicted the RAVLT A1 score. Independent of age, the number of years from the surgery was negatively correlated to the number of words recalled on the RAVLT ($B = -0.87, p < .01$) (Table 8). A plot of A1 performance versus time since oophorectomy suggests that women using ERT performed better than women without ERT (Figure 2).

Table 8

Summary of Regression Analysis for Variables Predicting RAVLT A1 (n = 9)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B$</td>
<td>$SE B$</td>
<td>$\beta$</td>
<td>$B$</td>
<td>$SE B$</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Education</td>
<td>4.121</td>
<td>0.143</td>
<td>-0.251</td>
<td>0.069</td>
<td>0.084</td>
<td>0.184</td>
</tr>
<tr>
<td>Age</td>
<td>-0.094</td>
<td>0.419</td>
<td>0.374</td>
<td>0.571</td>
<td>0.220</td>
<td>0.514</td>
</tr>
<tr>
<td>CES-D</td>
<td>0.061</td>
<td>0.274</td>
<td>0.079</td>
<td>-0.146</td>
<td>0.150</td>
<td>-0.188</td>
</tr>
<tr>
<td>TSS</td>
<td>0.061</td>
<td>0.274</td>
<td>0.079</td>
<td>-0.146</td>
<td>0.150</td>
<td>-0.188</td>
</tr>
<tr>
<td>$R^2$</td>
<td>-.082</td>
<td></td>
<td></td>
<td>.711</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F$ of $R^2$ Change</td>
<td>.772</td>
<td></td>
<td></td>
<td>17.49**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Time since surgery (BSO); **$p < .01$

Figure 2. Time since BSO was a negative predictor of performance on the RAVLT A1, which assesses verbal short-term memory.
3.6 Effect of the APOE4 Allele on the RAVLT
An independent t-test revealed a significant difference on the RAVLT Recency subscale between carriers and non-carriers, which measures the ability to recall recently learnt items ($t(20) = 2.13, p = .046$). On average, ε4 carriers regardless of experimental group (mean = 11.67, SD = 1.97) recalled the last three words of the RAVLT less frequently than non-carriers (mean = 13.25, SD = 1.39).

3.7 Effect of Year of Testing
As there were only two participants in the BRCA group who have completed their second year of testing, there was insufficient data to perform an analysis to determine the effect of year of testing as a function of group. A repeated measures ANOVA using on the six BSO participants who have completed two years of testing revealed a marginally significant effect of the number of words produced in the Phonemic component of the Verbal Fluency task ($F(1,5) = 6.19, p = .055$). In the second year of testing, a mean of 15.4 words (SD = 2.9) were produced - a decrease from the mean of 17.3 words (SD = 4.0) in the first year.

3.8 Non-significant results
Many tests did not yield significant results (Table 9). For our secondary hypotheses, ANCOVAs were used to test group differences, multiple regressions used to test E1G and time since oophorectomy as predictors of cognitive outcome measures, and t-tests used to test differences between E4 and non-E4 carriers. Preplanned comparisons between AMC and BSO groups were also conducted with t-tests.
Table 9

Summary of all statistical tests conducted

<table>
<thead>
<tr>
<th>Measure</th>
<th>Hypothesis</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LM Immediate &amp; Delayed</strong></td>
<td>BRCA ≠ AMC ≠ BSO</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>SPWM total errors</strong></td>
<td>E1G positive $^1$</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Digit Span Total</strong></td>
<td>E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>RAVLT A1</strong></td>
<td>Time since BSO correlates negatively to performance</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>Non-ε4 &gt; ε4</td>
<td>n.s.</td>
</tr>
<tr>
<td>No. of semantic words</td>
<td>BRCA ≠ AMC ≠ BSO</td>
<td>n.s.</td>
</tr>
<tr>
<td>Semantic cluster size</td>
<td>BRCA ≠ AMC ≠ BSO</td>
<td>n.s.</td>
</tr>
<tr>
<td>Semantic switches</td>
<td>BRCA ≠ AMC ≠ BSO</td>
<td>n.s.</td>
</tr>
<tr>
<td>No. of phonemic words</td>
<td>BRCA ≠ AMC ≠ BSO</td>
<td>n.s.</td>
</tr>
<tr>
<td>Phonemic cluster size</td>
<td>BRCA ≠ AMC ≠ BSO; year of testing</td>
<td>n.s.</td>
</tr>
<tr>
<td>Corsi Blockspan</td>
<td>BRCA ≠ AMC ≠ BSO</td>
<td>n.s.</td>
</tr>
<tr>
<td>DOT total errors</td>
<td>E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>RAVLT A6</td>
<td>E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>RAVLT B1</td>
<td>E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>RAVLT Best Trial</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>RAVLT A total</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>RAVLT Learning</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>RAVLT Primacy</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive; Non-ε4 &gt; ε4</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>RAVLT Recency</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive; Non-ε4 &gt; ε4</td>
<td>&lt;.05$^A$</td>
</tr>
<tr>
<td>RAVLT Proactive Int.</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive</td>
<td>.054</td>
</tr>
<tr>
<td>RAVLT Retroactive Int.</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>RAVLT Interference</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>OPT Imm. Displacement</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>OPT Del. Displacement</td>
<td>BRCA ≠ AMC ≠ BSO; E1G positive</td>
<td>n.s.</td>
</tr>
<tr>
<td>MRT</td>
<td>E1G correlates negatively to performance</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Notes. Primary hypotheses are indicated in bold. If there were multiple hypotheses per measure, the one underlined corresponds to the p-value reported.

$^1$E1G correlates positively to performance; $^A$E1G was a negative predictor of performance
Chapter 4

4 Discussion

In this study, the effects of 17B-estradiol-deprivation and APOE carrier status were studied using a battery of neuropsychological tests assessing a range of cognitive functions. Women with BSO performed worse on a test of episodic verbal episodic memory (LM) compared to those with intact ovaries. As well, E1G predicted performance of verbal short-term memory (RAVLT Primacy) in women with BSO. Our findings are generally consistent with those previously reported (Sherwin and Phillips, 1992; Farrag et al., 2002). An important and novel result that emerged in this study was that performance on the RAVLT A1 decreased as a function of time since oophorectomy even after controlling for age. Results based on primary hypotheses will be discussed first, followed by a discussion of secondary hypotheses.

4.1 Primary Hypotheses

4.1.1 There will be group Differences in Logical Memory.

There is considerable support in the literature that verbal, episodic memory is a sensitive test following oophorectomy (e.g. Sherwin, 1988, 1992; Farrag et al., 2002), hence this was an effect we expected to observe. Pre-planned comparisons confirmed that the BSO group recalled the fewest details on both stories – significantly fewer than the AMC group on both immediate and delayed conditions. Although we also predicted a difference at the level of the three experimental groups, this hypothesis is currently not supported by the data. The lack of group differences is likely due to the large variability in the data and the low numbers of participants in the BRCA and BSO groups. In spite of this, pairwise comparisons indicate that the data conform to previous findings that women with BSO perform significantly worse than age matched controls on paragraph recall. Since this is an effect that has been robustly reproduced, a difference between BSO and AMC groups will likely persist as more data are collected.

4.1.2 Estrogen-deprivation will affect performance on SPWM.

The SPWM task tests frontal cortical function and in two papers previously published, performance was found to be positively correlated in E1G (Duff & Hampson, 2001; 2000);
however, we found that estrogen was not a significant predictor of SPWM errors. Specifically, Duff and Hampson (2000) found that menopausal non-users of ERT made the most number of WM errors, followed by mixed formulations of estrogen only, and estrogen and progestins. Nevertheless, there is also evidence of increased spatial working memory errors with higher levels of estrogen in animals. Bimonte-Nelson et al. (2003) tested the performance of rats ovariectomized (OVX) at 22 months on the radial water maze task, reporting that OVX rats outperformed the aged surgical shams, at 1.5, 2, and 6 months post-surgically. Estradiol and progesterone were significantly lower the aged OVX rats, and the authors suggest the decrease of progesterone may have driven the enhanced spatial WM. To further investigate that progesterone may be related to spatial WM errors, Bimonte-Nelson et al. (2004) tested OVX rats on the radial-arm maze that were implanted with subcutaneous pellets releasing 30mg of progesterone per day. As hypothesized, this counteracted the previously reported enhancement of spatial WM after OVX. When we looked at PdG and performance on the SPWM task, PdG was a marginally significant predictor of errors on the first trial of the SPWM task ($B = 0.327, p = .066$), indicating increased error with PdG concentration. Because PdG was only a marginally significant predictor of SPWM errors, it remains inconclusive whether higher levels of progesterone are associated with impaired spatial working memory.

4.1.3 Estrogenic Effects on Digit Span.

While estrogen was not a predictor in the Digit Span task, interpretation of results is limited by the lack of data. With only nine women in the BSO group, the recommended 15 data points per predictor in multiple regression was not satisfied (Stevens, 1996, p. 72). The Digit Span task (DS) is intended to assess attention and working memory (Hale et al., 2002). Phillips and Sherwin (1992) failed to find a difference on the DS task pre, and post-oophorectomy in 19 women regardless of whether they received a two-month period of 10mg E$_2$ valerate treatment or the placebo. However, a prior study by Sherwin (1988) indicated that 44 surgically menopause women who were treated with estrogen, androgen, or an estrogen-androgen combination performed significantly better on the DS test seven months after oophorectomy compared to the placebo. Similarly, Farrag et al. (2002) found a significant decline on the DS in 35 women three, and six months post-BSO. Although a post-operative correlation between performance and serum estrogen levels was not tested, a significant decrease in estrogen levels was observed at
three months, and a yet greater decrease after six months. Thus, Farrag et al. (2002) produced indirect evidence that lower estrogen levels may be related to poorer performance on the DS.

4.1.4 Effect of Time since Oophorectomy on the RAVLT.
Controlling for the effects of age, the number of words recalled on the A1 of the RAVLT decreased with time since BSO, suggesting it is an effect of time since surgical menopause. The mean score of females between 40 and 49-years-old on the RAVLT A1 is 6.6 (SD = 1.5) (Strauss, Sherman & Spreen, 2007), and it appears that during first year post-BSO, individual performances exceed 6.6 but decreases with years since oophorectomy (Figure 2). The predictive value of time since oophorectomy on RAVLT A1 has been a particularly robust effect in this study that previously has emerged with a smaller dataset, and this effect will likely persist in the next iteration of data analyses. However, it should be noted that certain subscales have greater reliability: the sum of the A trials, A5, and A6 (Strauss, Sherman & Spreen, 2006), and our primary hypothesis should perhaps be altered to reflect this. In particular, the RAVLT A6 (short delayed recall) subscale has 70% sensitivity and 73% specificity in predicting the development of dementia up to 10 years before a clinical diagnosis is made (Tierney et al., 2005), and making it a contender to replace the RAVLT A1 as an outcome measure in our primary hypotheses.

4.2 RAVLT
A marginally significant group difference was found on the RAVLT Proactive Interference subscale. Nonetheless, the lack of significant difference between the AMC and BSO participants suggests that the marginally significant result at the group level was perhaps due to age. The Proactive Interference subscale measures an individual’s ability to “free up” their memory in order to attend to, and learn new stimuli (Groth-Marnat, 2003). Because the ANCOVA did not reach statistical significance even before correction for multiple comparisons, this effect may be lost with a larger sample size.

It was also the case that some of the changes found on the RAVLT depend on estrogen levels. The level of E1G in the BSO group was found to be a significant predictor of the RAVLT Primacy subscale. This scale is scored as the number of times participants recall the first three
words of the A trials on the RAVLT and thus, related to memory performance. Tierney et al. (1994) have reported utility of the serial position effects of the RAVLT in distinguishing normal ageing from AD in septuagenarians, with controls recalling the first and last five words of the RAVLT more often than patients with mild to severe AD. Further, patients with AD recalled significantly more words towards the end of the list than the beginning.

In addition, we found that estrogen levels were negatively predictive of the RAVLT Recency subscale; lower levels of estrogen were associated with a greater frequency of recalling the last three words across the first five RAVLT A trials. To date, there are no studies in the literature suggesting that estrogens affect the RAVLT Recency subscale. Because multiple hypotheses are being considered, results were not strictly significant unless they reached $p < .0083$, and it is possible that this reflects only a false positive at this point.

### 4.3 APOE

We also found that E4 carriers performed significantly worse on the RAVLT Recency subscale. According to Tuminello & Han’s theory of antagonistic pleiotropy (2011), the effects of E4 are neutral or positive before the age of 60. However, the instance of head trauma in younger E4 carriers is associated with poorer outcomes (Teasdale, Muray & Nicoll, 2005). While we do not currently have sufficient numbers in the BRCA control group to compare whether APOE affects the groups differently, our results suggest that BSO may be equivalent to an injury or illness that would shift the early benefits of the APOE4 allele to the older aged detrimental effects. This finding suggests that the RAVLT serialization effects are robust indicators of cognitive decline, or that once again, our dataset on APOE relative to cognitive performance is too small for results to be interpreted meaningfully.

### 4.4 Null and Marginally Significant Findings

While no group differences or estrogenic effects were found on the Phonemic and Semantic Fluency task in the current results, a performance decrement may become apparent in women further out from their BSO. Neither fluency tasks have been used in studies of cognitive changes following oophorectomy, but a meta-analysis reveals mostly a null effect in studies in
menopausal women (Henderson & Sherwin, 2007). In women between 70 and 78-years-old, hormone users performed significantly better than those who had never used ERT on the verbal fluency task (Grodstein et al., 2000), suggesting that estrogens may be associated with a decreased risk of later cognitive decline.

There was also a marginally significant difference found between the number of words produced on the Phonemic Fluency task in the six BSO participants, but the FAS (year 1) and CFL (year 2) forms differ in difficulty (Barry, Bates & Labouvie, 2008). The decrease in the number of words produced in the second year compared to the first should therefore be attributed to this difference in difficulty rather than interpreted as a veridical performance decrement. Age and years of education were found as significant predictors of performance of the Verbal Fluency task, suggesting that in future analyses, participant age and education should remain as a covariate.

Currently, we observe neither an effect of group, nor estrogen on Petrides’ DOT (1993), a particularly demanding task of verbal WM which we would expect based on Petrides et al. (1993) report of increased cerebral blood flow to the mid dorso-lateral frontal cortex as participants performed the DOT. ERα has been localized to this region as well (Bixo et al., 1995). On another working memory task in 11 naturally menopausal women, Duff and Hampson reported fewer errors in HRT-users (a combination of estrogen and progesterone) compared to non-users. Although we did not replicate this finding, it is notable that estrogen levels in our BSO group were highly varied due to a number of participants using ERT. Therefore, a group difference in working memory may be found once our dataset is large enough to afford the removal of BSO participants using ERT.

We also did not observe an effect of estrogen on performance on the Corsi Block Tapping task. This is a visuospatial analog of the Digit Span task included because we do not expect to find it affected. Two studies to date have reported a null effect of estrogen on the CBT in naturally menopausal women: there was no difference on the CBT between women using HRT (Duff and Hampson, 2001) or in women receiving a 16 week supplementation of soy isoflavone, a compound chemically similar to 17β-estradiol (Fournier et al, 2007). Likewise, there are also no reports of sex differences on the CBT in adolescents (Smimi, Villardia and Zappala, 1983) or undergraduates (Postma et al., 2004). Based on the literature, it appears that estrogens do not
particularly affect performance on the CBT, and building on this, we do not expect one to be found in our study.

We did not find any group differences or estrogenic effects on the Object Placement Task. The OPT has not been used in the assessment of cognitive changes after BSO, but spatial ability has been explored extensively in the context of sex differences, which many believe depend on differences in estrogen levels between women and men. Whether a sex difference exists in spatial memory is disputed because it comprises several, distinct processes including object-to-position assignment, positional reconstruction, and integration (Postma, Izendoorn & de Haan, 1998). Studies of spatial memory may tap into one, or a combination of these processes. Thus, in a task similar to the OPT, where participants were shown 10 objects arranged on a square display on the computer for 30s, and asked to replicate their arrangement, no sex difference was found in absolute errors (Postma, Izendoorn & de Haan, 1998). The OPT relies on all three processes, and it remains to be seen whether estrogenic effects will emerge as there are data supporting both female (e.g. Silverman & Eals, 1994) and male advantage (e.g. Miller and Santoni, 1987) in spatial memory.

Contrary to the findings of others, we did not observe an effect of estrogen on the MRT, such that lower levels were beneficial to performance. Mental rotation ability has been linked to the menstrual cycle, and women perform optimally during menses when estrogen levels are lowest (Silverman & Phillips, 1993). During menses, the mean score on the MRT was almost twofold the score obtained when participants were not menstruating. Sex differences in spatial ability are well established in the literature, with males outperforming females on mental rotation tasks (Voyer, Voyer & Bryden, 1995). This superiority has recently been qualified to tasks with an active processing component (Vecchi & Girelli, 1998). Since most of the literature suggesting an estrogenic effect on the MRT, we may not see one due to the current lack of power. The adaptation of the Vanderberg and Kuse (1973) Mental Rotation task used in this study was a particularly challenging task for participants – many of whom expressed a poor self-assessment of mental rotation ability. The MRT was introduced into our battery approximately a year ago and only eight participants at time one have been tested to date. Due to the low estrogen bias on active spatial tasks, we expect that women with BSO will perform better on this task than either AMC or BRCA controls.
That the cognitive reserve of an individual can buffer against decline is a possible reason why we did not observe the hypothesized differences between the BSO and AMC groups on the majority of tasks at this stage. This concept is often discussed in the context of preclinical AD, and refers to factors lending to an asymptomatic clinical presentation, despite neurophysiological changes detectable by imaging or at autopsy. Cognitive reserve is associated with years of education, intelligence and bilingualism (Valenzuela & Sachdev, 2006), which is relevant to our sample of well-educated participants. An individual may exhibit a substantial amount of neuropathologies, yet do not degrade in a neuropsychological assessment for a number of years. For instance, Ince (2001) reported that approximately 25% of elders who exhibited AD pathology upon autopsy were not impaired on neuropsychological measures pre-mortem. On the premise that neuropathologies may occur prior to behavioural changes, a neuroimaging extension of this study is in the early stages of planning. The goal of this arm will be to detect the earliest anatomical or functional differences between BSO women and their age-matched cohort, in order to determine if and when brain changes map onto compromised cognitive changes.

4.5 Limitations

As this study is in its preliminary stages, perhaps the most apparent limitation is the lack of statistical power with the amount of data collected at this time. It was estimated 193 participants in total will be needed to support our primary hypotheses with 80% power. Values of current effect sizes and the method of estimating the required N are presented in Appendix D.

Another limitation is that we have not been able to schedule controls for testing during the same time of their menstrual cycles when hormone levels would be equivalent. We had previously intended to test everyone during menses because estrogen levels would be lowest then. However, due in large to the scheduling difficulties, it was not possible at this time for BRCA and AMC participants to be tested uniformly during the week of their menses, in order to reduce the variation in E1G levels. Of those willing to volunteer their time, availability was limited. As such, the correlation between E1G and cognitive performance were only calculated for the BSO. Nevertheless, isolating the effects of E1G to women with BSO has allowed the quantification of how varying levels of E1G supplemented through ERT, or synthesized by adipose tissue and
adrenal glands can contribute to cognitive functions.

Ultimately, because the purpose of this study is to investigate how estrogen deprivation following surgical menopause affects cognition, women using ERT should not be grouped together with non-users. The inclusion of ERT users to the dataset at this point was necessitated by the small number of BSO participants. As more participants are recruited, it will be possible to assess whether the use of ERT is beneficial over time for women with BSO, and how carrying the APOE4 allele affects women if they are using ERT.

The current inclusion of two control groups does not address two theoretical concerns: (1) how BSO women compare to those who are naturally menopausal, and (2) whether the BRCAm itself may affect cognition. By including naturally menopausal women of the same age, the difference between the effects of surgical and natural menopause on cognition could be identified. As for whether the BRCAm affects cognition, while it has been extensively studied in the context of familial gynecological cancers, there is no research to date on how mutations may affect cognition. In addition to an increased risk of gynaecological cancers, a germline mutation of BRCA1 is associated with immunodeficiency (Zielinski et al., 2003). There is no data suggesting either BRCAm affects cognition, but as DNA repair genes, their effects in the body are ubiquitous. We found that performance on the LM by the BRCA group was intermediary, despite an expected advantage due to age (Figure 1). Inclusion of a control group in the same age range as the BRCA carriers who have not yet elected BSO may help to determine whether the BRCA mutation also affects cognitive function.

A related concern stems from the effects of surgery, which might warrant the addition of a surgical control group. In the present study, no one was tested until at least six months post-surgery, a window of time that should be ample for patients to equilibrate. As BSO is a minor surgery typically performed laparoscopically, and patients report a similar quality of life before the surgery and one year after (Finch et al., 2011), we do not expect the surgery itself to have a physically traumatic effect. In support of this, one study has included a hysterectomy control group, which indicated a null effect of a urogenital operation on cognitive function (Sherwin, 1988).
Lastly, the current dataset included two participants with a history of breast cancer despite evidence of cognitive changes related to chemotherapy in breast cancer patients. “Chemo brain” is a well-known phenomenon, referring to a general state of mental fog, with symptoms such as memory lapses, difficulty attending, or anomia manifesting during, or after cancer treatment (American Cancer Society, 2012). Putative mechanisms by which chemotherapy may be associated with cognitive impairment include “neurotoxic effects…oxidative stress and DNA damage [and] induced hormonal changes…” as well as exacerbating a genetic predisposition (Vardy et al., 2008, p. 625). Interestingly, carrying an apoE4 allele has been found to pose increased vulnerability to outcomes of chemotherapy. Ahles (2003) reported that breast cancer survivors who had received chemotherapy who were also E4 carriers scored significantly lower on measures of visual memory and spatial ability as assessed by the Visual Reproduction task and Block Design task of the WAIS. Notwithstanding chemotherapy, there is evidence that cognitive dysfunction occurs prior to initiation of cancer treatment in some patients with breast carcinoma or lymphoma. Neuropsychological testing of 84 women with breast cancer before receiving adjuvant therapy revealed that performance was impaired in 35% of the cohort, particularly in verbal learning and memory (Wefel et al., 2004). Although “chemo brain” may only be transient (Schagen et al., 2002), caution dictates that in the future, data collected from patients with a history of cancer should be treated separately.

### 4.6 Conclusion

The strength of this study lies in the longitudinal design, and focus on the BRCAm population, an important group in which the cognitive outcomes following oophorectomy have not been studied. With three observations per participant, modeling the data with an accelerated growth curve will allow us to observe the trajectory from one to ten years post-oophorectomy. Although small scale RCTS (e.g. Sherwin, 1992) have demonstrated a difference pre- and post-surgery, this investigation endeavours to examine the trajectory of cognitive changes that may lead to dementia. The preliminary result that short-term memory (RAVLT A1) decreases as a function of time since oophorectomy reinforces the importance of understanding cognitive changes in otherwise healthy women post-surgically.

In closing, our preliminary results are in concert with the literature that there are changes to
verbal memory post-oophorectomy. This result and the epidemiological evidence that BSO prior to natural menopause is associated with a higher risk of dementia underscores the need for research investigating whether estrogen replacement post-BSO will be beneficial. A concern impeding such a study is the possible increase in risk of breast and ovarian cancer in women who already carry a high risk. However, one prospective study with 155 BRCAm carriers found that the use of ERT does not increase the risk of breast and ovarian cancer (Eisen et al., 2008; Rebbeck et al., 2005). In light of the results of this study suggesting some aspects of cognition decline post-BSO, it may be beneficial for younger women who are surgically menopausal to use ERT in order to mitigate the effects of estrogen deprivation. Further, if the BRCA mutation affects short-term memory - this disadvantage may be exacerbated by the loss of endogenous E2. Whether ERT can mitigate cognitive decline following oophorectomy is an area of clinical significance, and must be investigated much more extensively with longitudinal RCTs.
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## Appendices

### A. Medical History

Table 10

History of cancer, chemotherapy, and current medications of select participants

<table>
<thead>
<tr>
<th>Group</th>
<th>Cancer</th>
<th>ERT/OC</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSO</td>
<td>None</td>
<td>5</td>
<td><strong>Estradot</strong> (x3 – 17β-estradiol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Ortho 777</strong> <em>(norethindrone &amp; ethinyl estradiol)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Conjugated equine estrogens (625mg)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Concerta (27mg) for ADHD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fosamax (70 mg) for prevention of osteoporosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prozac (40mg)</td>
</tr>
<tr>
<td>AMC</td>
<td>None</td>
<td>0</td>
<td>Apo divalproex (250mg) for anti-seizure</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MaxGXL (gluthathione accelerator)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Asacol for colitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Celebrex for pain in limbs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tegretol (200mg) for epilepsy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nasonex (nasal steroid decongestant)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reactin for allergies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cymbalia (90mg) for mild depression</td>
</tr>
<tr>
<td>BRCA</td>
<td>Breast cancer (x2)</td>
<td>1</td>
<td><strong>Tri-cyclen</strong> <em>(norgestimate &amp; ethyl estradiol)</em></td>
</tr>
<tr>
<td></td>
<td>One participant received chemotherapy:</td>
<td></td>
<td>Cipralex (10mg)</td>
</tr>
<tr>
<td></td>
<td>Apr to Jul 07</td>
<td></td>
<td>Cymbalta (30mg) for neck pain and stress</td>
</tr>
<tr>
<td></td>
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<td>Tylenol 3 for mastectomy pain and migraines</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Levothyroxin for hypothyroidism</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>Levimir for Type 1 diabetes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Axert for migraines</td>
</tr>
</tbody>
</table>

*Notes.* Estrogen replacement medications and hormone oral contraceptives are indicated in bold.

\(^1\)There were four users of ERT and one user of oral contraceptives (OC) in the BSO group, and 1 user of OC in the BRCA group.
B. Results Excluding Users of ERT and Oral Contraceptives

After removal of four users of ERT and two users of estrogen-progesterone oral contraceptives, the data set was reduced to 31 participants (Table 11). There were no significant differences across the experimental groups on all demographic variables, except for age ($F(2,28) = 1.89, p = .01$). Once again, participants in the BRCA group were on average, younger than members of the BSO or AMC groups.

Table 11

*Characteristics of participants excluding users of ERT and oral contraceptives*

<table>
<thead>
<tr>
<th></th>
<th>BSO (n = 5)</th>
<th>AMC (n = 21)</th>
<th>BRCA (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>46.00</td>
<td>44.81</td>
<td>38.20</td>
</tr>
<tr>
<td><strong>IQ</strong></td>
<td>111.42</td>
<td>112.63</td>
<td>112.51</td>
</tr>
<tr>
<td><strong>Education (years)</strong></td>
<td>15.80</td>
<td>19.10</td>
<td>17.00</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>24.68</td>
<td>24.49</td>
<td>24.95</td>
</tr>
<tr>
<td><strong>E1G (ng/mL)</strong></td>
<td>12.46</td>
<td>32.96</td>
<td>32.19</td>
</tr>
<tr>
<td><strong>PdG (µg/mL)</strong></td>
<td>0.63</td>
<td>2.35</td>
<td>1.86</td>
</tr>
<tr>
<td><strong>CES-D Score</strong></td>
<td>6.20</td>
<td>6.24</td>
<td>2.60</td>
</tr>
</tbody>
</table>

Results were computed as described in the methods of the body with the exception of two differences: the treatment of outliers and variables controlled for in the regression models. First, removal of outliers based on the criteria of observations two standard deviations above or below the mean was not reapplied to the reduced dataset. In addition, regression analyses were conducted controlling only for age and years of education due to limitations by the number of data points. It was confirmed that CES-D scores were not significantly correlated to any of the measures that are reported as significant herein.

Differences by Group.

An ANCOVA revealed a marginally significant group difference on the Proactive Interference subscale (the difference between the RAVLT A1 and A6), controlling for age and years of
education ($F(2, 25) = 2.93, p = .07$). A significant difference between the AMC and BSO groups was found with a post-hoc t-test ($t(8) = 2.71, p = .034$), indicating the difference between the number of words recalled on A1 and A6 was greatest in the BSO women (Table 11).

An ANCOVA also revealed a significant group difference between the RAVLT Interference subscale, calculated as the difference between trials A6 and A5 ($F(2,24) = 5.23, p = .013$). A negative score indicated that participants recalled more words on A5 than A6, after learning the distracter list. No difference was detected between the AMC and BSO group with a post-hoc t-test ($t(8) = 0.42, p = .684$), suggesting this group difference was driven by the BRCA group and likely an effect of age (Table 11).

Table 11

*Descriptive statistics of the RAVLT subscales: Proactive Interference (n = 31) and Interference (n = 30)*

<table>
<thead>
<tr>
<th></th>
<th>BSO</th>
<th>AMC</th>
<th>BRCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Proactive Int.</td>
<td>-5.80</td>
<td>1.79</td>
<td>-3.81</td>
</tr>
<tr>
<td>Interference</td>
<td>-1.80</td>
<td>1.30</td>
<td>-1.45</td>
</tr>
</tbody>
</table>

Differences by E1G.

Controlling for years of education and age, E1G was a significant predictor of performance on the RAVLT B1 trials (Table 12). Higher levels of E1G positively predicted a greater number of words remembered on the new list of fifteen words ($B = 5.13, p < .05$).
Table 12

Summary of regression analysis for variables predicting B1 on the RAVLT (n = 5)

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
<td>β</td>
<td>B</td>
<td>SE B</td>
<td>β</td>
</tr>
<tr>
<td>Education</td>
<td>.242</td>
<td>.668</td>
<td>.215</td>
<td>2.12</td>
<td>.126</td>
<td>1.88</td>
</tr>
<tr>
<td>CESD</td>
<td>.441</td>
<td>.496</td>
<td>.259</td>
<td>1.03</td>
<td>.062</td>
<td>5.13</td>
</tr>
<tr>
<td>E1G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>.416</td>
<td>.</td>
<td>.998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F of R² Change</td>
<td>.713</td>
<td></td>
<td>159.72*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05

Figure 3. E1G versus number of words recalled on the RAVLT B1 subscale.

Time since BSO.

Time since BSO made a significant contribution to the OPT difference (B = -0.948, p < .05) (Table 13). An average displacement value was produced in the OPT by subtracting the position of each object the participant reproduced on the corkboard from its veridical position. The OPT difference was then calculated by subtracting the average displacement on the immediate difference from the average displacement on the delayed condition. Time further out from oophorectomy was predictive of a smaller difference between the delayed and immediate conditions of the OPT (Figure 3).
### Table 13

*Summary of regression analysis for variables predicting the OPT Difference (n = 5)*

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
</tr>
<tr>
<td>Education</td>
<td>-257</td>
<td>0.267</td>
</tr>
<tr>
<td>CES-D</td>
<td>-0.096</td>
<td>0.199</td>
</tr>
<tr>
<td>TSS(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R(^2)</td>
<td>.481</td>
<td></td>
</tr>
<tr>
<td>F of R(^2) Change</td>
<td>.928</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)TSS: time since oophorectomy; *p < 0.05

---

**Figure 4.** Years since oophorectomy versus the OPT difference.

---

**C. Discussion Excluding Users of ERT and Oral Contraceptives**

Excluding users of ERT and oral contraceptives, the group difference on the RAVLT Proactive Interference subscale (the difference between the A1 and A6 trials) was now marginally significant. The mean score of the BSO group was largest in magnitude in the negative direction, indicating that on average, they recalled more words on A6 after being read the list of words five times, compared to the A1 trial. This can be interpreted as the BSO group recalling fewer words.
on the A1 trial, or more words on the A6 trial. Upon examination, the BSO group recalled on average the fewest words on A1 and A6 (Table 12). Mirroring the results found including ERT-users, there was no difference between the AMC and BSO groups. At present, it can only be concluded that the experimental groups differ on this measure as a whole, but it is not driven by a difference between women with oophorectomies and their controls per se.

A group difference was also found on the RAVLT Interference subscale, calculated as the difference between the A6 and A5 trials. It is possible that this group difference was not significant when users of ERT and oral contraceptive were included because these participants introduced considerable variability to performance. This is seen by comparing the standard deviations of RAVLT Interference in Table 11 and Table 14. On average, the Interference score of the BRCA group was closer to 0 than either BSO or AMC group, indicating they produced approximately the same number of words on A5 and A6 after learning the distractor list. Age was likely driving this difference because the independent t-test comparing the BSO and AMC was non-significant.

In this subset of the data, E1G was a significant predictor of performance on the RAVLT B1, with higher levels associated with the ability to recall more words. The B1 trial, known as the distractor list, added fifteen new words to the pre-existing memory load of participants. Our results suggest that higher levels of E1G post-BSO were associated with better recall ability despite increased demands. The mean score in our sample population (n = 5) was 6.2, which was on par with the mean score of 5.9 (SD = 1.9) in healthy adult females between 40 and 49-years-old (Strauss, Sherman & Spreen, 2006, p. 787). However, one participant with undetectable levels of E1G (i.e. 0 ng/mL) scored the lowest on the RAVLT B1, and those with intermediary levels of E1G clustered around a score of 6, suggesting a single participant may have driven this result. In the full dataset (n = 37), E1G was positively associated with performance on the B1 as well, but did not reach statistical significance (B = 0.602, p = .14). An alternative interpretation is that lower levels of E1G may be associated with verbal memory regardless of their source.

Time since oophorectomy was a significant predictor of the OPT difference excluding users of ERT and oral contraceptives. For participants further out from BSO, the displacement from the delayed condition did not increase as much from the intermediate condition as it did in
participants who had their surgery more recently. Results suggest a greater amount of time elapsed since BSO is predictive of a smaller change in the spatial representation of the board from the immediate, to the delayed condition. Because one particular participant who was a year post-BSO scored 3.31 on the OPT difference whereas the four remaining participants ranging one to seven years post-BSO scored 1.51 or less, it is possible that again, a single data point was driving the current results. With limited data, it cannot be ascertained if this participant was simply an outlier. Alternatively, there may be in fact an improvement in spatial memory further out from BSO. Including the women on ERT and oral contraceptives, a similar trend was observed but did not reach statistical significance \( B = -0.519, p = .129 \). As discussed, studies in OVX rats have indicated superior spatial memory compared to surgical shams (e.g. Bimonte-Nelson, 2004) – hence an improvement in spatial memory following oophorectomy in women is plausible.

### D. Descriptive Statistics of all Measures by Group

Table 14

Mean scores on all measures, and standard deviations (in parentheses)

<table>
<thead>
<tr>
<th>Measure</th>
<th>BSO</th>
<th>AMC</th>
<th>BRCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. phonemic words</td>
<td>17.43 (3.79)</td>
<td>16.61 (4.23)</td>
<td>15.33 (2.74)</td>
</tr>
<tr>
<td>Phonemic cluster size</td>
<td>0.50 (.25)</td>
<td>0.76 (1.28)</td>
<td>0.22 (0.16)</td>
</tr>
<tr>
<td>Phonemic switches</td>
<td>12.37(2.67)</td>
<td>10.92(3.53)</td>
<td>11.89 (0.94)</td>
</tr>
<tr>
<td>No. semantic words</td>
<td>22 (4.95)</td>
<td>21.15 (5.52)</td>
<td>22.33 (4.27)</td>
</tr>
<tr>
<td>Semantic cluster size</td>
<td>0.85 (0.39)</td>
<td>0.95 (0.37)</td>
<td>1.05 (0.51)</td>
</tr>
<tr>
<td>Semantic switches</td>
<td>11.4 (3.34)</td>
<td>10.30 (3.08)</td>
<td>11.33 (2.50)</td>
</tr>
<tr>
<td>Logical Memory Immediate A</td>
<td>9.56 (2.40)</td>
<td>12.10 (4.34)</td>
<td>12.00 (4.56)</td>
</tr>
<tr>
<td>LM Immediate B</td>
<td>6.10 (2.42)</td>
<td>7.78 (2.92)</td>
<td>7.17 (2.79)</td>
</tr>
<tr>
<td>LM Immediate Total</td>
<td>15.00 (3.80)</td>
<td>19.89 (6.09)</td>
<td>19.17 (5.91)</td>
</tr>
<tr>
<td>LM Delayed A</td>
<td>6.80 (3.12)</td>
<td>9.42 (4.39)</td>
<td>8.67 (4.93)</td>
</tr>
<tr>
<td>LM Delayed B</td>
<td>5.40 (2.17)</td>
<td>6.06 (2.36)</td>
<td>5.80 (1.30)</td>
</tr>
<tr>
<td>LM Delayed Total</td>
<td>12.20 (3.49)</td>
<td>16.12 (4.92)</td>
<td>15.50 (4.89)</td>
</tr>
<tr>
<td>SPWM Time 1 (s)</td>
<td>251.67 (105.65)</td>
<td>225.37 (76.34)</td>
<td>233 (61.69)</td>
</tr>
<tr>
<td>SPWM Time 2 (s)</td>
<td>210.20 (80.13)</td>
<td>213.20 (82.05)</td>
<td>194.6 (67.60)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPWM Trial 1 errors</td>
<td>35.3 (13.78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPWM Trial 2 errors</td>
<td>45.9 (10.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPWM Total Errors</td>
<td>81.50 (19.55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span Forward</td>
<td>6.67 (1.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span Backwards</td>
<td>4.50 (1.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span Total</td>
<td>11.33 (2.24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corsi Block Span</td>
<td>6.00 (0.94)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corsi Total Span</td>
<td>55.20 (17.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAVLT A1</td>
<td>7.2 (1.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAVLT A2</td>
<td>10.50 (1.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAVLT A3</td>
<td>13.38 (1.41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAVLT A4</td>
<td>13.00 (1.58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAVLT A5</td>
<td>14.50 (0.76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAVLT B1</td>
<td>12.44 (1.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAVLT A6</td>
<td>6.20 (1.81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best Learning Trial</td>
<td>14.33 (1.12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total A</td>
<td>59.60 (13.91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Learning over Time</td>
<td>19.89 (6.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Learning</td>
<td>6.00 (1.87)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retroactive Interference</td>
<td>1.80 (1.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proactive Interference</td>
<td>-4.80 (1.62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primacy</td>
<td>12.22 (0.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recency</td>
<td>12.33 (1.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interference</td>
<td>-1.60 (1.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Ordering Task mean time (s)</td>
<td>13.85 (4.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOT repetitions</td>
<td>3.80 (2.62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOT omissions</td>
<td>7.10 (4.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOT total errors</td>
<td>10.90 (4.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPT Immediate disp. (cm)</td>
<td>5.58 (0.88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPT Delayed disp. (cm)</td>
<td>7.29 (1.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPT disp. difference (cm)</td>
<td>1.71 (0.88)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
D. Estimation of Required Sample Size

To limit the possibility of a Type I error, six hypotheses have been selected using the Logical Memory, Digit Span, and RAVLT. Effect sizes and estimated sample sizes were calculated using G*Power (3.1), with power ($1-\beta$) set as 0.80 and $p < .0083$ with the current dataset of 37 participants (Table 15). Factoring in the effect of the APOE gene based on existing literature was problematic due to the small effect size of the $\varepsilon 4$ allele on cognition of individuals without dementia (Small et al., 2004). Rather than claim a significant difference will exist between carriers and non-carriers (i.e. with a t-test), it may be more practical to assert $\varepsilon 4$ carriers will perform worse than average using a sign test. Estimated effect sizes for hypotheses 4 and 5 were generated with a sign test; the N was initially computed by G*Power then divided by 0.145 to account for the frequency of the apoE4 allele.

Table 15

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Effect size</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Group differences in LM immediate total</td>
<td>$N_p^2 = 0.14$</td>
<td>93</td>
</tr>
<tr>
<td>2. E1G is a predictor of Digit Span forward</td>
<td>$f^2 = 0.09$</td>
<td>135</td>
</tr>
<tr>
<td>3. E1G is a predictor of SPWM errors (excluding ERT users)</td>
<td>$f^2 = 0.11$</td>
<td>113</td>
</tr>
<tr>
<td>4. RAVLT A1 decreases by time since oophorectomy</td>
<td>$f^2 = 0.85$</td>
<td>19</td>
</tr>
<tr>
<td>5. $\varepsilon 4$ carriers are worse on RAVLT Retroactive Interference</td>
<td>$g = 0.40$</td>
<td>97</td>
</tr>
<tr>
<td>6. $\varepsilon 4$ carriers are worse on RAVLT B1</td>
<td>$g = 0.30$</td>
<td>193</td>
</tr>
</tbody>
</table>

Notes: $N_p^2$: the amount of variance accounted for by the predictor variable  
$f^2 = proportion of the variance explained by predictor, out of all the unexplained variance  
g = deviation from the expected frequency (50%) at which $\varepsilon 4$ carriers should be worse than average