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Lifetime Estrogen Exposure, COMT Genotype, and Cognition in Postmenopausal Women

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Abstract

In the future, it may be possible to slow the change in cognition associated with menopause. First, we must understand the mechanism with which different biological processes interact in order to slow this decline at the source. An important gene that plays a role in cognition and aging is the gene for catechol-o-methyltransferase, which is an enzyme that degrades dopamine in the prefrontal cortex (PFC). Different genotypes cause expression of varying amounts of the enzyme intended to degrade dopamine in the synapse. When estrogen is present in the system, transcription of COMT is inhibited. The cognitive effect of the interaction between lifetime estrogen exposure and COMT was examined in 65 healthy postmenopausal women. In this study, we tested episodic and working memory, which are associated with PFC functioning. The subjects were genotyped for the COMT SNP (single nucleotide polymorphism). An index of lifetime estrogen exposure (ILEE) was created that incorporated reproductive period (menarche to menopause), total duration of breast-feeding, total time on hormonal therapy, and time since menopause. We found that in working memory and episodic memory measures, the effects of ILEE on cognition depended on the COMT genotype. This study showed that cognition in women with the lower dopaminergic baseline gene, Val/Val, benefitted from lifetime estrogen exposure. However, cognition in women with the higher baseline dopamine gene, Met/Met, was negatively affected by lifetime estrogen. Women with the Met/Val gene were not as impacted by lifetime estrogen. These results suggest that the COMT gene should be taken into account when considering exogenous estrogen to modulate the decline of cognition after menopause.

Keywords: estrogen, menopause, COMT, prefrontal cortex, dopamine, cognition

Introduction

Menopause may be the most important biochemical change in a woman's life. During this period the noticeable effects on the brain vary greatly. Some women experience a decline in memory and other cognitive measures, while others experience very little pathological change. With the onset of menopause, cognitive decline may be accelerated (Halbreich et al., 1995a). This acceleration might be due to a deficiency of gonadal hormones, including estrogen. It is important to understand the underlying neurobiological and genetic mechanisms of menopause and aging.

Aging causes a decrease in the availability of dopaminergic receptors in the brain, shown using positron emission tomography (Volkow et al., 1998; Wong et al., 1984). Dopamine is a neurotransmitter that is involved in cognition and the functioning of the dopaminergic system changes in normal aging (Braver and Barch, 2002). Dopamine is synthesized in the substantia nigra and the ventral tegmental regions (VTR) of the brain. From the VTR, it follows the mesocortical pathway to affect cognitive processes, circulating through the striatum and to the prefrontal cortex (Malenka et al., 2009).

The concentration of dopamine in the prefrontal cortex (PFC) is controlled by the COMT gene, which encodes for the COMT enzyme (Lachman et al., 1996). Although the primary site of the COMT enzymatic interaction with dopamine occurs in the PFC, this enzyme is widely expressed throughout the brain. The region where the highest dopamine or dopamine metabolite concentration is the basal ganglia, specifically the lateral putamen, along with high levels found in the caudate nucleus and the nucleus accumbens (Hall et al., 1994). In regions like these, the dopamine transporter (DAT) is more likely to control dopamine concentrations since the DAT has a thousand times greater dopamine affinity then the COMT enzyme (Lewis et al., 2001).

However, PFC dopamine levels are unaffected by DAT since the DAT is minimally expressed in this area (Huotari et al., 2002; Sesack et al., 1998). This means the COMT enzyme is the primary mediator of dopaminergic functioning in the PFC. In addition, mice COMT knockout studies suggest that dopamine concentration was substantially greater in the PFC compared to the striatum, where the dopamine transporter mainly controls dopamine functioning (Gogos et al., 1998). Further research shows that COMT mRNA levels are greater in the PFC than the striatum (Matsumoto et al., 2003). Therefore, COMT primarily mediates prefrontal cortex dopamine levels rather than other brain areas, suggesting that COMT controls PFC derived cognitive processes, including working memory.

This COMT gene has a SNP, which determines COMT enzymatic activity, with the Met allele having about a quarter of the dopamine degrading enzymatic activity of the Val allele (Chen et al., 2004). Dopamine impacts cognitive functioning related to the PFC, which includes working memory, attentional control, and episodic memory (Egan et al., 2001; Schott et al., 2006). Cognition is impacted differently when different concentrations of dopamine are present. Specifically, the dopamine receptor produces an inverted-U dose-response, with too little or too much dopamine impairing working memory (Vijayraghavan et al., 2007). It is important to understand the factors that impact dopamine concentration.

Human dopamine functioning undergoes dynamic changes through development, affecting the male and female brains differently (Schulz et al., 2009). In addition, an animal model of primates suggested that females experience a decline in working memory with the onset of menopause, independent of age (Hara et al., 2014). Both of these findings imply that the dopaminergic system is affected by sex hormones, which readily cross the blood-brain barrier.

Research shows that estrogen's role in cognition is primarily mediated by the PFC and its circuitry (Keenan et al., 2001; Krug et al., 2006). Specifically, estrogen has a direct effect on the COMT gene by inhibiting transcription when estrogen is bound to both response elements on the promoter of the gene (Weinshilboum, 2006; Xie et al., 1999). Evidence suggests that estradiol increases dopamine activity (Thompson and Moss, 1994) and dopamine synthesis, release, and turnover (Becker, 2000). Therefore, the COMT genotype and exposure to estrogen both affect the amount of dopamine in the brain, which in turn impacts cognition.

One study on premenopausal women found cognitive changes based on the interaction between COMT and estrogen levels during the menstrual cycle (Jacobs and D'Esposito 2011). Moreover, this study determined that optimal dopaminergic system functioning occurred in the Met/Met genotype, but the Val/Val genotype combined with high estrogen during the menstrual cycle showed the greatest effect on brain functioning during a working memory task. This finding demonstrates that the changes in hormone levels during the menstrual cycle interact with the COMT genotype to influence performance and brain functioning during a working memory task.

Many studies examined the cognitive influence of exogenous estrogen use during or after menopause, each with varying results (Carlson et al., 2001; Lethaby et al., 2008; Maki, 2005; Ryan et al., 2009). There are several theories for these inconsistencies. One is that a "critical window" of estrogen exposure during menopause increases neuroprotection, and different ages of hormone therapy (HT) may be the reason for variation in the results. Another theory is that genetic components in the brain impact the range of cognitive results from HT. Lastly, lifetime estrogen exposure might modulate the effects of HT through menopause. In order to test the lifetime estrogen theory, studies have explored cognitive changes associated with lifetime

estrogen exposure, but these results are varied as well (Low et al., 2005; Tierney et al., 2013). Tierney et al. (2013) found that the reproductive period (between menarche and menopause) length was related to better delayed visual memory, immediate and delayed verbal memory, and working memory. On the other hand, Low et al. (2005) determined that the reproductive period length had no detectable effect on cognition. These studies had not taken into account the COMT genotype, which may be one reason for the varying results. No one has studied the cognitive effects of lifetime estrogen with the COMT genotype in postmenopausal women.

Studies often used different markers to identify cumulative estrogen exposure. Several well-researched factors cause changes in estrogen levels throughout a woman's life. Exogenous gonadal steroid use through hormonal contraceptives directly increases estrogen levels. The duration and age of use of exogenous gonadal steroids could have different effects on postmenopausal cognition. There are also many endogenous factors that influence hormones. The duration in a woman's life when she is reproductive, marked by age at menarche and age at menopause, influences serum estrogen levels (Geerlings et al., 2001). Pregnancies increase short-term estrogen levels because the placenta synthesizes and releases estrogen into the body (Siiteri and MacDonald, 1966). Levels of estrogen are also influenced by interruptions in pregnancies, whether they are spontaneous abortions (Coulam and Stern, 1994) or induced abortions (Daling et al., 1996). The Body Mass Index (BMI) of an individual also increases estrogen in the body (Lukanova et al., 2004). The duration of breast-feeding postpones ovulation due to inhibition of FSH stimulation in the ovary, and has been shown to depress plasma estrogen levels (Bonnar et al., 1975).

Rather than examining these lifetime estrogen factors individually, it has been shown that combining multiple markers into one index increases analytical power and makes results more

apparent (Smith et al., 1999). Smith et al. (1999) combined several estrogen markers into a simple Index of Estrogen Exposure (IEE). Factors with clear effects on estrogen were used in this index. The IEE excluded most reproduction factors because although pregnancy considerably increases short-term estrogen levels, the long-term implications are more complicated. Research suggests that parous women experience a gradual decrease in estradiol level with age during the follicular phase until menopause (Dorgan et al., 1995). Conversely, follicular phase estradiol levels increase with age in nulliparous women prior to menopause. To construct the IEE, Smith et al. (1999) standardized the data, then added time on postmenopausal estrogen therapy, age at menopause, nulliparity, and postmenopausal weight. Age at menarche and time since menopause were subtracted. This study found that the IEE was most strongly correlated with global cognitive functioning and verbal attention. No individual estrogen marker was related to cognition.

Another study used the IEE based on Smith et al. (1999) but included breast-feeding as a factor that decreased estrogen (Hesson, 2012). They called this index the Index of Cumulative Estrogen Exposure (ICEE). No significant relationship was found between ICEE and retrospective memory, but the data suggested prospective memory was positively correlated with ICEE. In the current study, two indexes of estrogen exposure were used: one that measured every variable recorded in the questionnaire (TLEE), and another based on the research of Smith et al. (1999) and Hesson (2012), using only markers that clearly increased estrogen (ILEE). The main difference between these two created indexes is that the TLEE includes total duration pregnant and hormonal contraceptives, while the ILEE takes into account years since menopause onset.

The current study examined the interaction between the lifetime estrogen exposure in postmenopausal women and functioning of the dopamine system in the brain. The purpose of

this study was to examine how lifetime estrogen exposure interacted with the COMT gene to affect cognition after menopause, which no other study has examined. Depending on how the cognitive performance of women with different genotypes is affected by lifetime estrogen, there may be different hormonal treatments or lifestyle choices suggested in the future. It was hypothesized that a longer period of exposure to estrogen had a greater effect on cognition in women with lower dopaminergic functioning, the Val/Val genotype of COMT, more than women with higher dopamine functioning, with Val/Met and Met/Met genotypes.

Methods

Participants

Women were recruited by calling past research subjects of the Clinical Neuroscience Research Unit (CNRU) in the Department of Psychiatry and by posting advertisements around the community. These participants were recruited from the community, and the sample was not biased based on estrogen use. Once a potential participant called or answered the phone, these women were told about the study, and then answered questions about medical history during a telephone screening. Inclusion criteria were an age of 60 to 70, postmenopausal, and healthy. Women were excluded if they were current smokers, currently taking any medications that affected the central nervous system, such as anti-depressants, anti-anxiety medications, antihistamines, or medications altering the dopamine system. Medical history exclusions included diabetes, heart disease, cancer, chronic obstructive pulmonary disease, depression, anxiety, and a medical history of loss of consciousness for more than ten minutes due to head trauma. If a potential participant passed an initial telephone screening regarding age, medication use, disease history, and menopause history she was scheduled at the University of Vermont

Clinical Research Center (CRC) for one three-hour study visit. Once the participant arrived, authorization and consent forms were signed. Each woman provided a DNA sample, completed cognitive and behavioral screening tests, completed a questionnaire to disclose health information, answered questions about lifetime estrogen exposure, performed cognitive tasks to examine working memory and episodic memory, and filled out a series of questionnaires about subjective cognitive complaints. Details about each of these procedures are below

Cognitive Impairment Screening Tests

All subjects completed a set of initial cognitive status screening questionnaires: Mini Mental State Exam (MMSE) (Folstein et al., 1975), Dementia Rating Scale (DRS) (Jurica et al. 2001), and Brief Cognitive Rating Scale (BCRS) (Reisberg and Ferris, 1988) which lead to the Global Deterioration Score (GDS) (Reisberg et al., 1982). On both the DRS and MMSE, a higher score is related to better global cognitive functioning. Subjects were required to have a score equal to or greater than 26 on the MMSE, and a DRS score equal to or greater than 123. On the GDS and BCRS, a higher score was related to a greater amount of cognitive impairment. On the GDS, a score of 1 or 2 was required for eligibility.

Behavioral Screening

A series of behavioral screening questionnaires was used. This included a modified version of the Structured Clinical Interview for DSM-IV-TR (SCID) (First et al. 2001), which screened for current and past depression, current manic disorder, and current dysthymia. In addition, the Beck Depression Inventory (BDI-II) screened for current depression (Beck et al. 1996). In order to be considered depressed, a score higher than 9 on the BDI was used, along with an assessment of current major depressive disorder on the SCID. If a subject endorsed current depression on the SCID, her data were not included in analyses. None of the subjects in

the present study endorsed current depression, and thus were not ruled out due to the SCID. The Beck Anxiety Inventory (BAI) was used as a screen for anxiety (Beck et al., 1988). Subjects were disqualified from all analyses if a score above 15 was calculated from the BAI. One subject scored greater than 15 and her data were removed from the analysis. We used the Menopause Symptom Checklist to review the severity of menopausal symptoms the subjects experienced in the past month. This questionnaire was created by Newhouse and Sargent (2002), modeled after the Sherwin Menopause Index (Sherwin, 1991). Another questionnaire used for behavioral screening was the Pittsburg Quality Sleep Index (PQSI), which determined quality of sleep in the past month (Buysse et al., 1989).

Subjective Cognitive Complaints

Each woman filled out a series of questionnaires used to determine subjective cognitive complaints of subjects, the Cognitive Complaint Index (CCI). This battery included Memory Assessment Questionnaire (Pfeffer et al., 1982), Memory-Self Rating Questionnaire (Squire et al., 1979), Informant questionnaire on cognitive decline in the elderly (Jorm et al., 1994), and ten questions about mild cognitive impairment (Rabin et al., 2007). More subjective cognitive complaints had shown to be related to increased neurodegeneration (Saykin et al., 2006). These subjective cognitive complaints were not related to objective cognitive complaints, but were still related to brain functioning.

Cognitive Tasks

Working Memory – N-back Test

The N-back Test was used as a measure of verbal working memory. In this task, the subject viewed a string of consonant letters (except L, W, and Y), one every 3 seconds. Four conditions were presented: 0-back, 1-back, 2-back and 3-back. In each of the 1-back, 2-back, and

3-back conditions, the task was to decide whether the letter currently presented matched the letter that has been presented 1, 2, or 3-back in the sequence. The subjects were asked to press the "match" button when the letter on the screen matched the letter for the certain conditions, and the "mismatch" button for every other letter. In the 0-back condition, the subject was given a target letter and she made a "match" response when that target appeared. This condition required attention and focus, but no working memory. In the one-back condition, the goal was to press the match button when a letter matched the letter that appeared just prior (the letter appearing one item back). In the two-back condition, the match occurred when a letter was identical to the letter two items back. In the three-back condition, a match occurred when a letter was identical to the letter that appeared three items back. Participants were given two trial rounds of each condition before performing the full N-back task used in this study. Accuracy measures and reaction times were automatically recorded for each trial. Dumas et al. (2010) have shown that estrogen in postmenopausal women affected prefrontal brain regions involved in this task.

Episodic Memory Buschke Selective Reminding Test (SRT)

The Buschke SRT was used to measure episodic memory (Buschke and Fuld, 1974). The SRT is a multi-trial verbal list-learning task allowing the examination of acquisition, encoding, and retrieval. This standard test offers measures of storage into and retrieval from memory. For this task, a list of 16 unrelated words was read aloud to the subject. The subject recalled as many words as possible. Then, the person administering the task would selectively remind the subject of the words that she did not recall, and asked her to try to recall the list of 16 words again. There were eight trials in this task. In addition, there was one trail that was administered about 20 to 30 minutes after the end of the eighth trial. The total recall was the total amount of words the subject recalled from all eight trials. Consistency occurred when the subject remembered a word in succession for

two trials. Intrusions were words the subject recalled not on the specified list. Recall failure occurred when the subject failed to remember a word on two consecutive trials. Totals among these variables in all trials were added to give total recall, total consistency, total recall failure, total intrusions, and delayed recall.

Indices of Estrogen Exposure Questionnaire

Each woman answered questions about lifetime hormone exposure divided into 4 sections: menstrual cycle history, motherhood, menopause, and history of HT (Lord et al., 2009). The menstrual cycle history inquired about age at menarche, amenorrhea, and contraceptive hormone use. The motherhood section asked about pregnancies, miscarriages, and breast-feeding. The next section included age at menopause and whether it was natural or surgically induced. The last section was about hormonal therapy type, duration, and age. Lord et al. (2009) found this self-report estrogen use questionnaire to be reliable. The data collected from the questionnaire was converted into hormone history indices.

Index of Lifetime Exposure to Estrogen (ILEE)

All estrogen exposure markers were converted to years. Duration of breast-feeding was added across children for each woman, resulting in the total duration of breast-feeding. Time since menopause was calculated as current age minus age at menopause (age at last period). Adding all durations of hormonal therapy used for menopause created total hormonal therapy (total HT). To form the index, factors causing an increase in estrogen were added (age at menopause, total HT), and the markers that lead to a decrease in estrogen were subtracted (time since menopause, age at menarche, and total duration of breast-feeding). This index was based off of Smith et al. (1999) and Hesson (2012). However, unlike their models, I did not include postmenopausal weight (Smith) or BMI (Hesson) because this factor was an instantaneous

measurement rather than constant throughout life. I also did not include nulliparity in this index because the short-term and long-term effects of pregnancy on estrogen levels have shown to be contrasting (Dorgan et al., 1995).

Total Lifetime Estrogen Exposure (TLEE)

TLEE was an index that included all the variables on the questionnaire. Pregnancies were converted to years, and then added into the total duration pregnancy for each woman. Hormonal contraceptives were converted to years, creating the total hormonal contraceptives measure. The markers that cause an increase in estrogen were added (total duration pregnancy, total duration hormonal contraceptive, total duration hormonal replacement therapy, reproductive period from menarche to menopause) and one factor that is related to low estrogen (duration of breast-feeding).

Genotype

Subjects provided a buccal DNA sample. A clinical research nurse from the University of Vermont Medical Center, specifically the Clinical Research Center (CRC), ran a buccal cheek swab across the inside of each woman's cheek. Then, the sample was stored in a -20°F freezer until all the samples were collected. DNA extraction and single nucleotide polymorphism (SNP) analysis were conducted at the Vermont Cancer Center DNA Analysis lab. COMT val^{158}met polymorphism was determined using Taqman^{TM} Genotyping Master Mix. The PCR products were analyzed using Applied Biosystems Prism 7900HT Sequence Detection System, version SDS 2.4. Each woman was either Met/Met if there were two adenosine nucleotides at this loci, Met/Val if both alleles were present, or Val/Val if there were only guanine nucleotides.

Vital Signs and BMI

During each study day, a clinical research nurse from the CRC obtained height, weight, and the vital signs: respiratory rate, blood pressure, temperature, and pulse rate from the subject. Based on the height and weight, we calculated BMI for each subject.

Data Analysis

The COMT genotype was used as a grouping variable. In addition, a median split was used to separate women into high and low estrogen groups based on ILEE and TLEE separately. To test my hypotheses about lifetime estrogen exposure, COMT genotype, and cognition after menopause, I used a 2(ILEE: high and low) x 3(COMT genotype: Val/Val, Met/Val, Met/Met) ANOVA. ILEE group and genotype were between subjects factors. The ILEE hormone exposure variable was also used a continuous variable, changing the analyses into a regression model. Since the indices I used have many variable components, I also explored the correlations between cognition and the different markers for estrogen exposure individually. Many studies showed varying results in the literature of estrogen factors that were related to cognition.

Results

Participants

A total of 67 subjects were enrolled. Two subjects were excluded from analyses. One was ineligible because she was unable to finish the study day due to mental fatigue. The other reported above the allotted BAI score for anxiety. Out of the 65 analyzed subjects, 19 were Val/Val, 36 were Val/Met, and 10 were Met/Met. Table 1 shows the means of age, education, and BMI for the different genotypes.

Subjects ranged from 60 to 70 years old, with a mean age of 64.3 years (SD 3.1). These women had a mean education of 16.6 years (SD 1.9) and a mean BMI of 26.9 (SD 5.7). The average age at menarche was 12.7 years (SD 1.3) and the average age at menopause was 52.1 years (SD 3.3). In this study, 72.3% of women were parous, and, including pregnancy interruptions, like abortions and miscarriages, the mean total duration of pregnancy was 1.6 years (SD 1.1). Out of all the women analyzed, 61.5% breast-fed at least one child. 83.1% of the women reported using hormonal contraceptives, and the mean total duration of birth control for all women was 5.43 years (SD 6.4). In addition, 44.6% of the women used postmenopausal hormonal therapy throughout menopause, and among those, the mean duration of HT was 4.7 years (SD 5.1). Table 2 shows the averages for the estrogen markers measured. The means of the screening tests (Table 3), behavioral data (Table 4), and cognitive tasks (Table 5) are reported.

Working Memory (N-Back)

2-Back Hits, ILEE Grouping Variable, and COMT Genotype

The 2-Back condition measures working memory. In this condition, women were asked to indicate if the current letter matched the letter that appeared two items before.

There was an interaction between COMT genotype and the ILEE groups for the 2-back condition of the N-back working memory task ($F(2,59)=3.80$, $p=0.028$). There was also a main effect of COMT genotype ($F(2,59)=3.66$, $p=.032$). There was no main effect of the ILEE grouping variable ($p=.23$) (Table 6). Overall, the means showed the Val/Val group performed better than the Met/Val and the Met/Met groups. The *post hoc* *t*-test showed there was no significant difference between the Val/Val and the Met/Val groups ($t(53)=1.97$, $p= 0.054$) or between the Val/Val and Met/Met groups ($t(27)=1.52$, $p=0.140$). For the Val/Val group, the means showed that women in the low ILEE group performed worse than the women in the

Val/Val who were in the high estrogen ILEE group, but the *post hoc t*-tests were not significant ($t(17)=1.53$, $p=.143$). For the Met/Val group there was no difference between the high and low ILEE group ($t(34)=0.197$, $p=.845$). For the Met/Met group, there was a significant difference between the high and low ILEE group, with the low estrogen group performing better than the high estrogen ($t(8)=2.64$, $p=.030$). However, there were only two women in the high estrogen condition (see Table 7 and Figure 1).

3-Back Hits, ILEE Grouping Variable, and COMT Genotype

The 3-back condition is a more difficult task than the 2-back, as it requires holding more information in working memory.

There was no COMT genotype and ILEE group interaction for the 3-back condition ($p=.326$). However, there was a main effect of ILEE group ($F(2,59)=4.93$, $p=0.030$) and a main effect of COMT genotype for the 3-back of the N-back working memory test ($F(2,59)=4.00$, $p=0.024$) (Table 6). Overall the Met/Met group performed worse than the Met/Val ($t(44)=2.06$, $p=.045$) . The means showed that the Met/Met performed better than the Val/Val, but not significantly ($t(27)=1.56$, $p=.130$). There was no significant difference between the Met/Val and Val/Val groups ($t(53)=0.084$, $p=.934$). The means display the low estrogen performed better than the high estrogen group. There were no significant differences between high and low estrogen groups ($t(63)=1.34$, $p=.186$) (see Table 8 and Figure 2).

Episodic memory (SRT)

Total Recall, ILEE Grouping Variable and COMT Genotype

There was an interaction between COMT genotype and the ILEE groups for the total recall condition of the Buschke SRT for episodic memory ($F(2,59)=3.67$, $p=0.03$). There were no main effects of the COMT genotype ($p=0.12$) or the ILEE groups ($p=0.82$) (Table 6). For the

Val/Val women, the low ILEE group performed worse than the high ILEE group, but not significantly ($t(17)=1.85$, $p=0.081$). For the Val/Met women, high and low estrogen group performance did not differ much ($t(34)=0.270$, $p=0.789$). For the Met/Met group, the means indicated that low estrogen exposure group performed better than the high estrogen group, but not significantly ($t(8)=2.088$, $p=0.070$). (See Table 9 and Figure 3).

SRT Total Recall Failure, ILEE Grouping Variable, and COMT Genotype

There was an interaction between COMT genotype and the ILEE for the total recall failure condition of the Buschke SRT for episodic memory ($F(2,59)=7.22$, $p=0.002$). There were no main effects of the COMT genotype ($p=0.13$) or the ILEE groups ($p=0.42$) (Table 6). For the Val/Val women, the means showed that the low ILEE group had significantly more recall failures, denoting worse performance, than the high ILEE group ($t(17)=2.26$, $p=0.037$). For the Val/Met women, the high estrogen group and the low estrogen group had similar performance ($t(34)=0.666$, $p=0.510$). For the Met/Met group, the high estrogen exposure group had significantly more recall failures than the low estrogen group ($t(8)=4.40$, $p=0.002$) (see Table 10 and Figure 4).

Individual estrogen components correlate with cognition

When I used the ILEE as a continuous variable instead of grouping variable there was no correlation between estrogen exposure and working memory, episodic memory, or cognitive complaints. However, there was a correlation between global cognitive functioning measured with the DRS and continuous ILEE ($r= .330$, $p=.007$). Higher global cognitive functioning was related to greater lifetime estrogen exposure.

The ILEE factor has many components. Separately, some of these markers were related to cognition. Understanding which factors were related to cognition alone is very important and

many studies research individual components rather than making indices. Thus, I examined correlations between the individual hormone measures and the cognitive measures.

Global Cognitive Functioning

There was a correlation between global cognitive functioning measured with the DRS and the amount of time since menopause (r= -.368, p= .003). This means that less time since menopause was related to higher global cognitive functioning.

Subjective Cognitive Complaints

There was a correlation between the subjective cognitive complaints reported with the MFQ and the total duration pregnant (r=.416, p=.001). Thus, greater subjective cognitive complaints were related to a longer total duration pregnant.

Episodic Memory

There was a correlation between number of total intrusions, a measure of inaccurate recall, on the Buschke SRT and the total pregnancy duration (r=.357, p=.004). Therefore, more intrusions were related to longer total duration pregnant.

Age was an important factor among some variables of the Buschke test. Age was correlated with the Buschke task total recall (r= -.272, p=.029). This means that increased age was related to performing worse on the total recall on the Buschke SRT. Also, age was correlated to total consistency on the Buschke SRT (r= -.276, p= .026). Increasing age was related to worse performance on consistency of the Buschke SRT. Following this pattern, total recall failure on the Buschke SRT was correlated with age (r= .248, p= .046). Increasing age was related to more recall failures.

Working Memory

On the N-back task, 0-back hits were positively correlated with total menopausal hormone therapy duration (r=.286, p=021). This means that a longer duration of hormone therapy through menopause was related to better performance on the 0-back task. However, 0-back hits were negatively correlated with the duration of hormonal contraceptive earlier in life (r= -.321, p=.009). Therefore, the longer the duration of hormonal contraceptives was related to a worse 0-back performance.

For the 1-back condition on the N-back task, hits were negatively correlated with TLEE (r= -.29, p=.02). Higher total estrogen exposure, considering all the markers, was related to poor performance on the 1-back condition.

However, performance was close to ceiling for the 0-back and 1-back conditions.

Discussion

As hypothesized, the effects of lifetime estrogen exposure on cognition were modulated by COMT genotype, since a greater amount of lifetime estrogen exposure benefitted the lower baseline dopamine genotype (Val/Val), but was detrimental to women with the higher baseline dopamine gene (Met/Met). Overall, the means for working memory, as measured by 2-back hits, and the episodic memory measures, SRT total recall and SRT recall failure, suggested that the effects of ILEE on cognition depended on the COMT genotype. This study showed that cognition in Val/Val women was positively affected by lifetime estrogen exposure. However, cognition in women with the higher baseline dopamine gene, Met/Met, was more negatively affected by higher lifetime estrogen exposure. Women with the Met/Val gene displayed cognitive performance that seemed unaffected by lifetime estrogen. This is probably because in

the PFC, dopamine was most beneficial at an intermediate concentration, but more detrimental as it reached the extremes.

Interactions between genotype and estrogen affecting cognition were also found in an fMRI study of premenopausal women (Jacobs and D'Esposito, 2011). In the Jacobs and D'Esposito (2011) study, the effects of estrogen depended on the baseline of dopamine determined by genetics. The fMRI showed similar results to my study because Jacobs and D'Esposito found Met/Met women performed best under low estrogen conditions and decreased performance under high estrogen conditions. In addition, the Val/Val subjects had impaired cognition when estrogen was low, but improved performance with higher estrogen.

With the increased working memory load in the 3-back condition, the interaction of COMT and ILEE group on cognition no longer occurred. However, main effects of both genotype and ILEE were present. The ILEE main effect for the 3-back condition had means that displayed the low ILEE group performed better than the high ILEE group. The genotype main effect showed the Met/Met genotype did worse than both the Val/Val and the Val/Met. In addition, the greatest performance difference between high and low ILEE occurred in the Met/Met genotype, with the high ILEE Met/Met group performing worse than all other groups. When considering the main effects of ILEE and genotype separately, it appeared the women with higher dopamine performed worse. This might be because studies have suggested more dopamine correlated with increased anxiety, which is related to a decreased attention control that may have affected performance on the difficult 3-back condition (Eysenck et al., 2007). In addition, research suggested that anxiety is a characteristic of the high baseline dopamine allele, Met/Met (Olsson et al., 2005). An fMRI study suggested that due to the difficulty level, the 3-back condition caused more disengagement, and the 2-back was likely a better predictor of

working memory because most subjects can remain focused and with continual effort (Ayaz et al., 2012). This disengagement is likely seen in higher dopamine individuals more because of higher anxiety rates. Therefore, the 3-back condition might not be as predictive of cognitive performance as the 2-back condition.

In addition to the results of the results from the ANOVA, there was a correlation between ILEE as a continuous variable and global cognitive functioning. This is a replication of the finding by Smith et al. (1999), suggesting that increased global cognitive functioning is related to women with greater lifetime estrogen exposure. However, ILEE as a continuous variable was not correlated to working memory or episodic memory. When grouped by a median split into high and low ILEE, there were effects on working memory and episodic memory. Further research is necessary in order to understand these relationships.

Limitations

One limitation in this study is that the Met/Met high estrogen group had a sample size of two women. Both of these women performed similarly in episodic memory and working memory tasks so there was at least no outlier in their performance. However, the relationship between cognition, estrogen, and COMT genotype found in this small sample size may be unreliable. A larger sample size of Met/Met women is needed to confirm that these results are not due to chance. The total sample size is a limitation to this study. With a larger sample, statistical power would be greater. Additionally, the women in this study were well educated, Caucasian, and all from the Vermont area. This sample probably does not accurately represent the population of postmenopausal women. With a larger sample size and greater variance through the sample, our results would be more reliable.

A second limitation was that the estrogen questionnaire was retrospective. The data were collected solely by subjective memory, and thus may not represent the true levels of lifetime estrogen exposure. However, prior studies have shown that the duration of hormone therapy through menopause and type of menopause (surgery, natural) were highly correlated with medical record findings of these events (Colditz et al., 1987; MacLennan et al., 2006). Other factors were found to be reliable, but not necessarily accurate in retrospective events because not compared to medical records. These included age at menarche, number of pregnancies, duration of hormonal contraceptive, and duration of HT use (Lord et al., 2009). In addition, doses of hormonal contraception and HT were not included since these factors were often unable to be reported. In addition, diet and lifetime BMI may be important factors when considering lifetime estrogen exposure, but since this study is retrospective, this information may be inaccurate or unreliable. Collecting data from both the women and medical records would reduce the chance of inaccuracies in the estrogen exposure data.

Future Directions

This study examined working memory, which has primarily function of the frontal lobes (Goldman-Rakic et al., 2004; Kane and Engle, 2002), and episodic memory, which is primarily a function of the hippocampus (Burgess et al., 2002). The N-back task has been shown in fMRI studies to reliably activate the bilateral frontal lobes but also activates bilateral parietal lobes and bilateral cerebellum (Cohen et al., 1997). The Buschke is a measure of episodic memory, which primarily requires hippocampal functioning for good performance (Buschke and Fuld, 1974; Rosen et al., 2003). However, the frontal lobes are also involved in episodic memory performance (Wheeler et al., 1995). A future study might attempt to use cognitive tasks that require smaller or more prescribed brain networks for their performance. However, the current

tasks were chosen because of the reliable nature of the brain networks that they require for good performance. Some potential examples might be a test of spatial navigation to specifically involve the hippocampus or a category test or decision-making tasks (Shepard et al., 1961) to examine frontal lobe function further.

Conclusions

Many studies show that lifetime estrogen exposure increased cognition later in life (Heys et al., 2011; Li et al., 2014; Ryan et al., 2009; Silverman et al., 2011), although results varied (Low et al., 2005). Studies reported that estrogen modulates cognition by inducing dendritic spine formation of the CA1 region of the hippocampus in a temporary fashion (Woolley and McEwen, 1992). However, this finding does not support why lifetime estrogen would impact cognition in the PFC. One study explored the effects of estradiol on the PFC in female monkeys and suggested that that long term exposure to estradiol (17β) with cyclical peaks may be the most effective way to cause dendritic spine generation, turnover, and consolidation (Hao et al., 2006). Therefore, it is possible that lifetime estrogen, particularly the reproductive period from menarche to menopause, induces a permanent change in dendritic spines.

There are a number of potential mechanisms that may explain how lifetime estrogen affects cognition after menopause. Estrogen influences multiple neurotransmitters, including cholinergic (Bora et al., 2005; Luine, 1985), serotonergic (Halbreich et al., 1995b), adrenergic (Sar and Stumpf, 1981), and dopaminergic (Roy et al., 1990) systems. In addition, estrogen affects the brain in a number of ways. Estrogen is an endocrine sex hormone produced in the ovaries, which also functions in the central nervous system. Research has shown that estrogen can also be locally synthesized in the brain from cholesterol and act in as a paracrine hormone (Do Rego et al., 2009). This locally synthesized estrogen can regulate synaptic plasticity (Kretz

et al., 2004), as well as neurogenesis in the dentate gyrus (Tanapat et al., 2005). Estrogen can also be produced by neurons or astrocytes, or from circulating testosterone by aromatase. The aromatase level in the PFC was found to be significantly higher in females than males (Wei et al., 2014). Research shows that inhibited aromatase is related to memory impairment in females (Phillips et al., 2011). In an animal model, the brain-derived estrogen was a more direct and significant risk factor for cognitive decline by Aβ plaque formation than circulating estrogen (Yue et al., 2005). Therefore, more research is needed to determine the multiple effects of estrogen in the brain and how these are changed after menopause.

Another hypothesis of circulating estrogen is that telomerase plays a role in the endocrine-related cognitive decline. Longer telomeres are related to lower rates of cognitive decline and lower rates of cardiovascular disease (Miller et al., 2003). Estrogen can reduce oxidative stress and inflammation (Xing et al., 2009), which may cause a quicker decrease of telomere length (Correia-Melo et al., 2014; von Zglinicki, 2002). Overall, it has been shown that endogenous estrogen exposure, measured as reproductive years, protects against telomere shortening, and associated with higher cognition in older women (Lin et al., 2011).

Another possible mechanism by which lifetime estrogen impacts cognition after menopause is potentially by increasing cognitive reserve. Cognitive reserve is the idea that across the lifespan, higher education, participation in social or mentally stimulating activities, and complexity of occupation increases resistance to dementia (Harrison et al., 2015). Some studies show that women with higher levels of estrogen have better cognition (Sherwin, 1997). Perhaps compared to women with low levels of lifetime estrogen, women with higher lifetime estrogen continually displayed a better cognition, building better cognitive functioning leading

into older age. However, the neurobiological mechanisms underlying cognitive reserve remain to be determined.

The results of the current study suggest that women with a specific genotype would benefit more from estrogen therapy during menopause than others. In the future it may be possible to use a different form of medications to alleviate cognitive decline, such as a COMT inhibitor for Val/Val women. This may be more advantageous because higher levels of estrogen are related to an elevated risk of breast cancer (Colditz et al., 1993). In addition, some women have risk factors that are complications for exogenous estrogen due to medical contraindications or family history (Shifren and Schiff, 2010). More research with larger sample sizes about the interaction of lifetime estrogen, cognition, and genotype is needed before any treatment recommendations are made. However, the current data are a first step towards understanding these relationships.

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Table 1

Demographic data Means (standard deviation) by genotype

Genotype	Age	Education	BMI	N

Val/Val	64.4 (3.4)	17.3 (2.0)	24.7 (3.3)	19
Val/Met	64.2 (3.1)	16.3 (1.6)	27.7 (6.5)	36
Met/Met	64.1 (3.0)	16.2 (2.6)	28.6 (5.7)	10
Total	64.2 (3.1)	16.6 (1.9)	26.9 (5.7)	65

Table 2

Demographic and Lifetime Hormonal Exposure Means and

Standard Deviation

Estrogen Marker	Mean	SD
ILEE	30.1	6.9
TLEE	49.3	7.2
Age Menarche	12.7	1.2
Horm. Contraceptive Duration	5.4	6.4
Pregnancy Duration	1.6	1.1
Breast-Feeding Total Duration	.88	0.94
Age at Menonopause	52.1	3.2
HT Duration	2.0	4.0
Years Since Menopause	12.1	4.6

Note. ILEE=Index of Lifetime Estrogen Exposure; TLEE=Total Lifetime Estrogen Exposure; Horm.= hormonal

Table 3

Screening Questionnaire Means and Standard Deviation

Form	*Mean*	*SD*
MMSE	28.7	1.1
DRS	141	2.5
SCID- Past	0.2	0.4
BCRS	8.5	0.8
GDS	1.3	0.5

Table 4

Behavioral Data Means and Standard Deviation

Form	Mean	SD
BDI	1.3	2.1
BAI	2.6	3.1
Study Symptom	13.4	12.5
PQSI	4.1	3.2

Table 5

Cognitive Tasks/Complaints Mean and Standard Deviation

Measure	Mean	SD
SRT Total Recall	82.2	13.8
SRT Total Consistency	46.8	18.4
SRT Total Recall Failure	11.7	8.4
SRT Total Intrusion	1.5	2.2
SRT Delayed Recall	10.4	3.4
0 Back Hits	0.991	0.027
0-Back FA	0.002	0.007
1-Back Hits	0.93	0.134
1-Back FA	0.022	0.041
2-Back Hits	0.876	0.125
2-Back FA	0.102	0.063
3-Back Hits	0.731	0.155
3-Back FA	0.079	0.063
MFQ	5.29	1.05

Table 6

Results of Univariate Analyses of COMT genotype, ILEE, and the interaction between

COMT genotype and ILEE on working memory and episodic memory.

Dependent Variable	Source	*DF*	*F*	Sig.
2-Back Hits	Geno	2	3.66*	0.03
	ILEE	1	1.45	0.23
	Geno*ILEE	2	3.80*	0.03
3-Back Hits	Geno	2	4.00*	0.02
	ILEE	1	4.93*	0.03
	Geno*ILEE	2	1.14	0.33
SRT Total Recall	Geno	2	2.23	0.12
	ILEE	1	0.05	0.82
	Geno*ILEE	2	3.67*	0.03
SRT Total Recall Failure	Geno	2	2.11	0.13
	ILEE	1	0.67	0.42
	Geno*ILEE	2	7.22**	0.002

Note. *p < .05. **p< .01.

Table 7

Proportion 2-Back Hits Correct Split by COMT Genotype and ILEE Group

Geno	ILEE	Mean	SD	N

Val/Val	Low	0.881	0.156	9
	High	0.964	0.069	10
	Total	0.925	0.123	19
Met/Val	Low	0.862	0.128	15
	High	0.854	0.118	21
	Total	0.857	0.121	36
Met/Met	Low	0.893	0.108	8
	High	0.679	0.051	2
	Total	0.850	0.132	10
Total	Low	0.875	0.128	32
	High	0.877	0.124	33
	Total	0.876	0.125	65

Table 8

Proportion 3-Back Hits Correct and Standard Deviation Split by COMT Genotype and ILEE Group

Geno	ILEE	Mean	SD	N
Val/Val	Low	0.754	0.160	9
	High	0.736	0.196	10
	Total	0.744	0.175	19
Met/Val	Low	0.795	0.132	15
	High	0.714	0.141	21
	Total	0.748	0.141	36
Met/Met	Low	0.688	0.114	8
	High	0.464	0.152	2
	Total	0.643	0.147	10
Total	Low	0.757	0.139	32
	High	0.706	0.167	33
	Total	0.731	0.155	65

Table 9

SRT Total Recall Means and Standard Deviation Split by Genotype and Estrogen Group

Geno	ILEE	Mean	SD	N
Val/Val	Low	70.7	19.8	9
	High	84.5	12.3	10
	Total	77.9	17.3	19
Met/Val	Low	84.0	7.1	15
	High	85.1	14.5	21
	Total	84.6	11.8	36
Met/Met	Low	85.4	11.6	8
	High	67.5	2.1	2
	Total	81.8	12.7	10
Total	Low	80.6	13.9	32
	High	83.8	13.8	33
	Total	82.2	13.9	65

Table 10

SRT Total Recall Failure Means and Standard Deviation Split by Genotype and Estrogen Group

Geno	ILEE	*Mean*	*SD*	*N*
Val/Val	Low	19.0	13.3	9
	High	8.8	5.0	10
	Total	13.6	10.9	19
Met/Val	Low	9.7	5.6	15
	High	11.3	8.0	21
	Total	10.7	7.1	36
Met/Met	Low	8.8	4.5	8
	High	23.5	0.7	2
	Total	11.7	7.4	10
Total	Low	12.1	9.2	32
	High	11.3	7.6	33
	Total	11.7	8.4	65

Table 11

Correlations

	ILEE	TLEE	Age	T Dur Preg	Horm. Cont.	HT	Time Since Meno	SRT Intrusion	0-Back Hits	1-Back Hits	MFQ
TLEE	0.41*										
Age	-0.26	0.24									

Figure Legends

Figure 1. Mean proportion correct and standard error of 2-Back Hits split by genotype and Index of Estrogen Exposure (ILEE) group. There was an interaction between COMT genotype and the

ILEE groups for this task ($F(2,59)=3.80$, $p=0.028$). There was also a main effect of COMT genotype ($F(2,59)=3.66$, $p=.032$). For the Met/Met group, there was a significant difference between the high and low estrogen group with the low ILEE group performing better than the high ILEE group ($t(8)=2.64$, $p=.030$). $*p< 0.05$.

Figure 2. Mean proportion correct and standard error of 3-Back Hits Split by Genotype and Index of Estrogen Exposure (ILEE) group. There was a main effect of ILEE group ($F(2,59)=4.93$, $p=0.030$) and a main effect of COMT genotype for the 3-back of the N-back working memory test ($F(2,59)=4.00$, $p=0.024$). Overall the Met/Met group performed worse than the Met/Val ($t(44)=2.06$, $p=.045$). $*p< 0.05$.

Figure 3. Mean and standard error of SRT Total Recall Split by Genotype and Index of Estrogen Exposure (ILEE) group. There was an interaction between COMT genotype and the ILEE groups for the total recall condition of the Buschke SRT for episodic memory ($F(2,59)=3.67$, $p=0.03$).

Figure 4. Mean and standard error of Total SRT Recall Failure Split by Genotype and Index of Estrogen Exposure (ILEE) group. There was an interaction between COMT genotype and the ILEE for the total recall failure condition of the Buschke SRT for episodic memory ($F(2,59)=7.22$, $p=0.002$). For the Val/Val women, the low ILEE group had more recall failures, indicating worse performance, than the high ILEE group ($t(17)=2.26$, $p=0.037$). For the Met/Met group, the high estrogen exposure group had a greater amount of recall failures than the low estrogen group ($t(8)=4.40$, $p=0.002$). $*p< 0.05$.

Figure 1.

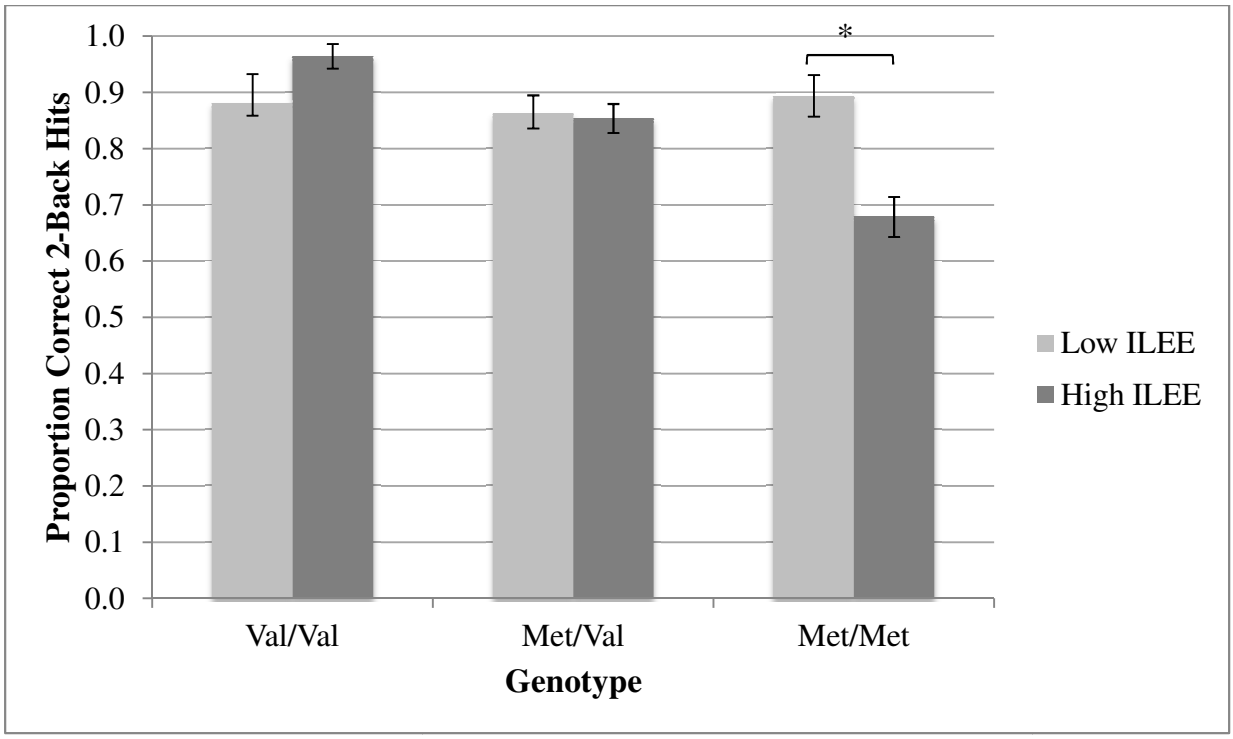

Figure 2.

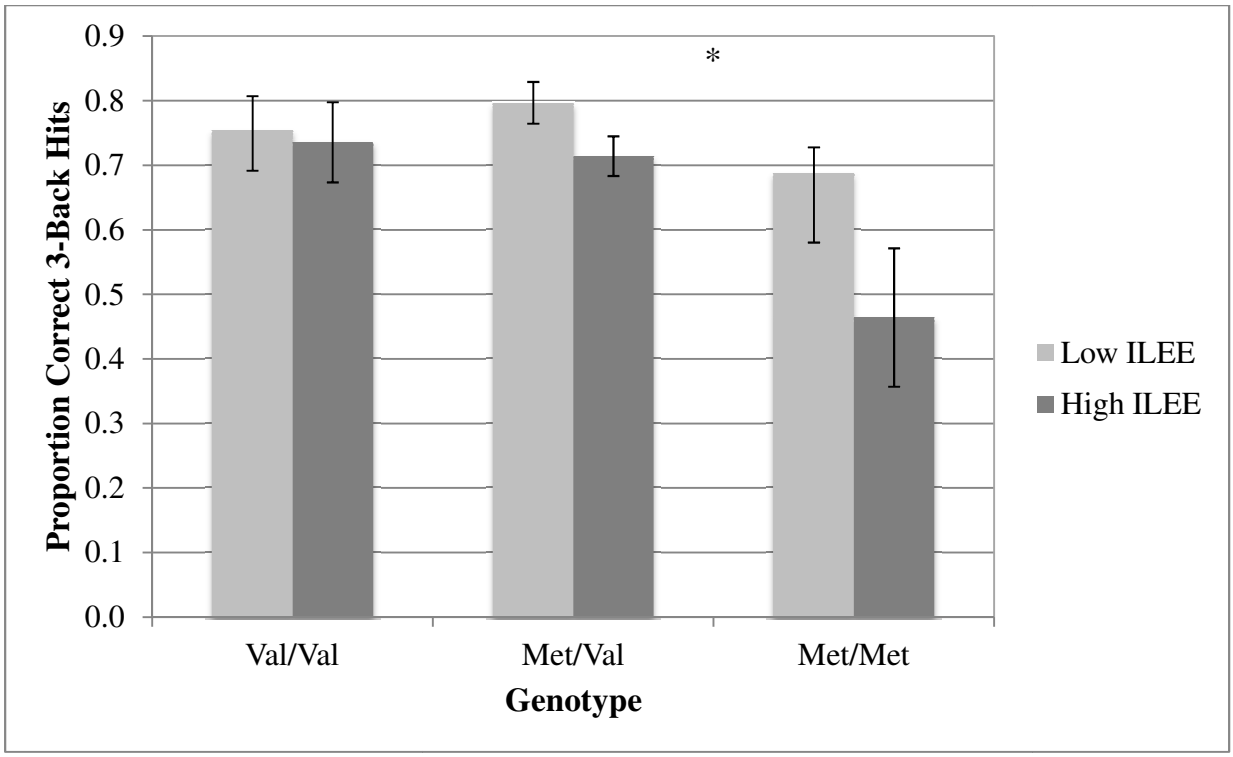

Figure 3.

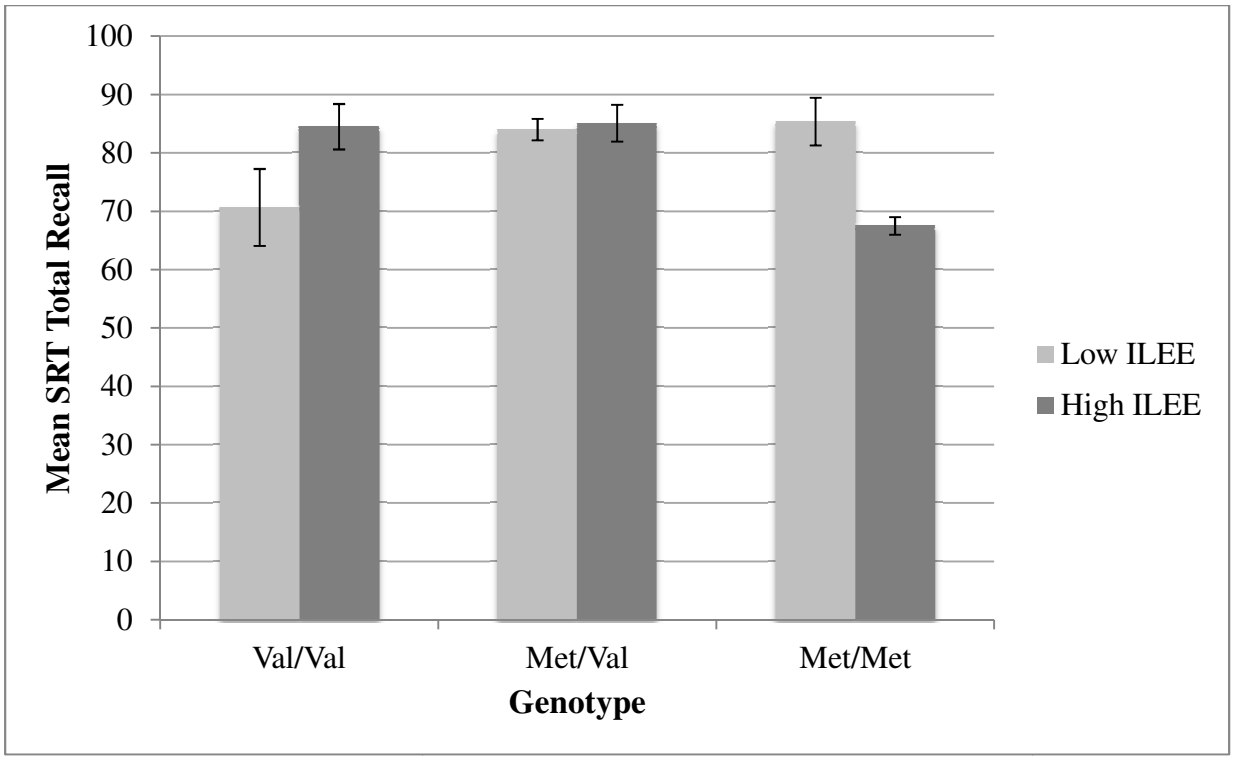

Figure 4.