Published in final edited form as:

Horm Behav. 2010 November ; 58(5): 929–935. doi:10.1016/j.yhbeh.2010.09.003.

Increased Memory Load-Related Frontal Activation after Estradiol Treatment in Postmenopausal Women

Julie A. Dumas^{1,*}, Amanda M. Kutz¹, Magdalena R. Naylor¹, Julia V. Johnson², and Paul A. Newhouse¹

¹ Clinical Neuroscience Research Unit and Brain Imaging Program, Department of Psychiatry, University of Vermont College of Medicine

² Department of Obstetrics and Gynecology, University of Massachusetts School of Medicine

Abstract

Prior research shows that menopause is associated with changes in cognition in some older women. However, how estrogen loss and subsequent estrogen treatment affects cognition and particularly the underlying brain processes responsible for any cognitive changes is less well understood. We examined the ability of estradiol to modulate the manipulation of information in working memory and related brain activation in postmenopausal women. Twenty healthy postmenopausal women (mean age (SD) = 59.13 (5.5)) were randomly assigned to three months of 1 mg oral 17- β estradiol or placebo. At baseline and three months later each woman completed a visual verbal N-back sequential letter test of working memory during functional magnetic resonance imaging (fMRI). The fMRI data showed that women who were treated with estradiol for three months had increased frontal activation during the more difficult working memory load conditions compared to women treated with placebo. Performance on the verbal working memory task showed no difference between estradiol and placebo treated subjects. These data are consistent with prior work showing increases in frontal activation on memory tasks after estrogen treatment. However, this is the first study to show that estrogen-induced increases in brain activity were tied to cognitive load during a verbal working memory task. These data suggest that estradiol treatment effects on cognition may be in part produced through modulation of frontal lobe functioning under difficult task conditions.

Keywords

working memory; estradiol; postmenopausal women; fMRI

During and after menopause women report complaints about changes in their cognitive functioning (e.g., Weber and Mapstone 2009). These changes are likely to be related to changing levels of steroid hormones after menopause and in particular the loss of estradiol. Research on the effects of estrogen and other hormone therapies on cognition after menopause has produced conflicting findings over the past 20 years. Data from the largest randomized clinical trial, the Women's Health Initiative (WHI) and Women's Health Initiative Memory Study (WHIMS), have shown that the risk of diagnosis of dementia in women over 65 taking combined estrogen and progesterone while small was twice that of women in the placebo group

*Address for Correspondence (JD): Clinical Neuroscience Research Unit, Department of Psychiatry, University of Vermont College of Medicine, 1 South Prospect St., Burlington, VT 05401, Voice: (802) 847-2523, Fax: (802) 847-7889, Julie.Dumas@uvm.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

while estrogen alone did not increase dementia risk (Shumaker et al. 2004; Shumaker et al. 2003). Additionally in the WHIMS study, estrogen plus progesterone did not improve cognitive performance and even resulted in an impairment relative to placebo treated women (Rapp et al. 2003). However, systematic review of studies since 2000 demonstrated that most clinical trials showed cognitive benefit of estrogen therapy in younger women but trials in older women showed no benefit or small negative effects (Maki 2005). Thus, there is evidence that hormone therapy after menopause may improve or maintain premenopausal levels of cognition however the conditions under which these benefits are observed are yet to be fully elucidated.

The current study examined the effects of estradiol on manipulation of information in working memory and related brain activation. Some prior studies have shown benefits of postmenopausal estradiol on working memory (Duff and Hampson 2000; Keenan et al. 2001), while combined estrogen and progesterone treatment showed a trend to impair working memory in the Women's Health Initiative Study of Cognitive Aging (WHISCA; Resnick et al. 2004). Examining hormone effects on brain circuitry involved in working memory processing may help in the understanding of hormone effects on cognitive processes. Three studies thus far have examined estrogen effects on working memory-related brain circuitry (Joffe et al. 2006; Shaywitz et al. 1999; Smith et al. 2006).

The first study conducted by Shaywitz et al. (1999) tested women during verbal and nonverbal working memory tasks after randomization to 21 days of 1.25 mg conjugated equine estrogen (CEE) or placebo. After a 14 day washout subjects received the other treatment for 21 days. Results showed that performance on the task was at ceiling, but there were differences in activation patterns between the estrogen and placebo phases. Estrogen increased activation in the left hemisphere during encoding into working memory and in the right superior frontal gyrus during retrieval. This was interpreted as producing a pattern of activation that is more similar to that seen in younger adults compared to older adults.

The two other studies examined the effects of hormones on short term retention of spatial information in working memory. In Smith et al. (2006) postmenopausal women were randomized to receive 5 µg ethinyl estradiol and 1 mg norethindrone acetate or placebo for four weeks. After a one month washout they were crossed over to the alternate treatment and scanned again. In Joffe et al. (2005) peri- and postmenopausal women were randomly assigned to receive 0.05 mg/day transdermal estradiol or matching placebo for 12 weeks. During the fMRI portion of both studies participants encoded spatial information into working memory. In both studies the results showed that activation was increased after hormone treatment compared to placebo in frontal regions. Specifically in Smith et al. (2006), Brodmann areas 44 and 45 showed greater activation during the delay period when subjects were holding information in working memory. In Joffe et al. (2006), there was greater activation in the superior frontal gyrus (BA 9) for the higher working memory load condition compared to the condition with less information to be remembered. Thus, frontal activation was increased in these studies when information was held in working memory.

While the working memory tasks used in prior studies required holding information in mind for a short period of time, these tasks did not require the active manipulation and constant updating of information in memory that are the hallmark cognitive components of a working memory task (Baddeley 1986; Just and Carpenter 1992). Studies of cognitive aging have shown that it is working memory tasks that require active manipulation of information are the ones that show the greatest age effects, with older adults performing more poorly than younger adults (see Bopp and Verhaeghen 2005 for a meta-analysis). Thus it is perhaps most relevant to understand the effects of estrogen on the working memory manipulation process that has been shown to be the most age sensitive and thus may be sensitive to hormonal changes at menopause. Behavioral studies have examined the effects of estrogen on the active

manipulation of information in working memory in postmenopausal women and have found benefits of estrogen treatment compared to placebo (Duff and Hampson 2000; Keenan et al. 2001). However the effects of estrogen on the neural correlates of active manipulation of information in working memory are not known.

The current study is part of a larger series of studies examining the effects of the interaction of estradiol and the cholinergic system in post menopausal women (Dumas et al. 2006; Dumas et al. 2008a). The focus of this paper is on the effects of three months of estradiol treatment on manipulation of information in working memory and related brain activation. The estradiol-cholinergic interaction data will be presented in a separate paper. We utilized the N-back sequential letter task that requires active manipulation and constant updating of information in working memory. Braver et al. (1997) and Cohen et al. (1997) initially showed that the frontal and parietal lobes were involved in active maintenance and updating of information during the N-back task.

One important benefit of the N-back task is that working memory load can be manipulated. In the current study, subjects performed the 0-, 1-, 2-, and 3-back conditions. This allowed us to examine the effects of estrogen as working memory load was increased from a condition with minimal memory load (the 0-back condition) to one in which subjects needed to hold four letters in memory and update working memory as the next letter in the sequence appeared (the 3-back condition). A prior study of older and younger adults showed that when older adults performed similar to younger adults in low working memory load conditions of 0- and 1-back, they had increased frontal activation (Mattay et al. 2006). However, when they showed impairments relative to younger adults in the 2- and 3-back conditions, older adults were unable to recruit brain regions to compensate for this impaired working memory performance. The current study examined the ability of estradiol to influence this working memory-related frontal activity in postmenopausal women. A prior study examining N-back performance after estrogen treatment showed that women taking estrogen had better performance across all N-back conditions and there was no interaction with working memory load (Keenan et al. 2001). However, the Keenan sample was a naturalistic sample and subjects were not randomly assigned to receive estrogen treatment.

In the current study, subjects were randomly assigned to take 1 mg per day of 17- β estradiol or placebo for three months. Subjects were scanned at baseline before the treatment period and again after the three month treatment. We hypothesized that three months of estradiol would increase frontal activation in all load conditions of the N-back task compared to three months of placebo.

Methods

Subjects

Subjects were 20 cognitively normal post-menopausal women (See Table 1 for demographic information). Four additional subjects passed the screening but withdrew before beginning hormone treatment because of the time commitment of the study. Subjects were randomly assigned to receive either three months of 1.0 mg oral 17- β estradiol (E2) per day or placebo. Demographic characteristics including age did not differ between the two groups (See Table 1).

Subjects were recruited through notices and advertisements in local newspapers and direct mailings. Subjects were required to be postmenopausal and without surgically-induced menopause. Exclusion criteria included use of tobacco or nicotine products within the last five years, a history of breast cancer, and use of hormone therapy during the last year. Twelve subjects had previously taken hormone or estrogen therapy after menopause. The length of

time of prior hormone use ranged from one week to 15 years ($M = 7.01$, $SD = 6.7$; See Table 1). There was no difference between the estrogen and placebo treatment groups on length of prior estrogen use. Medical exclusion criteria for E2 treatment included: contraindications for hormone therapy, estrogen-dependent neoplasia, untreated blood pressure greater than 160/100, history of deep vein thrombosis or other thromboembolic disease, hepatoma, severe migraines or stroke on oral contraceptives, current use of barbiturates, rifampin, insulin, carbamazepine, oral hypoglycemics, antidepressants, or lipid-lowering drugs, known intolerance to conjugated estrogens, diabetes, untreated thyroid disease, clinical osteoporosis, and a history or presence of severe menopausal symptoms. In addition, we also excluded women with a history of the following: heavy alcohol (more than an average of 1 drink per day) or coffee use (more than three cups per day), significant cardiovascular disease, asthma, active peptic ulcer, hyperthyroidism, pyloric stenosis, narrow angle glaucoma, epilepsy, or current Axis I psychiatric disorders. The alcohol criterion was used to ensure subjects were not alcohol abusing, and the caffeine criterion was used to ensure subjects would not experience caffeine withdrawal on testing days.

Upon meeting these criteria, subjects were approved for further screening at the University of Vermont (UVM) General Clinical Research Center (GCRC). After signing informed consent documents, subjects gave a medical history, underwent a physical and laboratory tests assessing hematopoietic, renal, hepatic and hormonal function. Subjects were cognitively evaluated using the Mini Mental State Exam (MMSE; Folstein et al. 1975), Brief Cognitive Rating Scale (Reisberg et al. 1988), and the Mattis Dementia Rating Scale (DRS; Jurica et al. 2001) to establish a Global Deterioration Scale score (GDS) which rated the degree of cognitive impairment (Reisberg and Ferris 1988). Subjects were required to have an MMSE score greater than or equal to 27, a DRS score of 123 or greater, and a GDS score of 1 or 2.

Behavioral screening consisted of a partial Structured Clinical Interview for DSM-IV-TR (SCID; First et al. 2001) to establish the presence/absence of Axis I psychiatric disorders. In addition, subjects completed the Beck Depression Inventory (BDI). A cut off score of 10 was used for the BDI, and subjects scoring over this criterion were discontinued from further participation. All subjects met these criteria for the cognitive and behavioral screening.

Estradiol Administration

After meeting all inclusion criteria, subjects were randomly and blindly assigned to the E2 or placebo condition for three months. In the E2 condition, subjects took 1 mg of oral 17-beta E2 per day for three months. In the placebo condition subjects took similar appearing placebo pills for three months. After three months of treatment estradiol levels were greater for the estradiol group, $M = 51.0$ pg/ml ($SD = 24.4$), compared to the placebo group, $M = 4.70$ pg/ml ($SD = 3.8$; $t(28) = 5.97$, $p < .001$). After three months, subjects completed three cholinergic challenge days (described below). After completion of the challenge days which took approximately three weeks, all subjects took 10 mg per day of medroxyprogesterone acetate for 12 days to produce sloughing of any endometrium that developed.

Challenge Procedure

After three months of estradiol or placebo treatment, subjects came to the UVM GCRC for three drug challenge days. These procedures are detailed in several prior papers and are not repeated here (see (Dumas et al. 2006; Dumas et al. 2008a; Dumas et al. 2008b)). The current study examined brain activation at baseline and during the placebo drug challenge day in order to assess the effects of estradiol treatment alone on brain activation. The challenge days were separated by a minimum of forty-eight hours to ensure complete washout of any study medication as was done in our prior studies. Two hours into the study day the fMRI session began with the visual verbal N-back.

fMRI Working Memory Task

We used a visually presented verbal N-back sequential letter task to probe working memory circuitry, wherein subjects saw a string of consonants (except L, W, and Y), presented in upper case letters, one every 3 seconds. Four conditions were presented: 0-back, 1-back, 2-back, and 3-back and on every trial subjects had to make a decision whether the current letter matched according to the rule which changed between blocks. Subjects completed 3 blocks of each condition that occurred in a pseudo-random order such that the same condition did not occur two times in row. Each block contained nine trials.

The 0-back control condition had a minimal working memory load; subjects were asked to decide if the current letter matched a single target letter that was specified before the block began. In the 1-back condition, they were asked to decide if the current letter matched the previous letter. During the 2-back condition, the task was to decide whether the letter currently presented matched the letter that had been presented two back in the sequence; the more difficult 3-back condition required subjects to decide if the current letter matched the letter three back in the sequence. Subjects responded to all items by button press through an MRI compatible fiber optic button response system (Eloquence System, Invivo Corp., Gainesville, FL) to indicate whether the item matched the target condition. Stimuli were delivered through an MR-safe computer monitor. Experimental tasks were presented by PC interface and were programmed using the E-Prime software package; the PC recorded subject responses and reaction times. The task ran for 8 minutes and 12 seconds.

fMRI Scan Procedure and Preprocessing

For logistical reasons, the first 11 subjects were scanned on one magnet while the last nine subjects were tested on a different magnet. The magnets were both Philips 3.0 Tesla Achieva scanners, all procedures and protocol files were the same on each magnet, and the same stimulus delivery and response equipment was used throughout the whole study. A comparison of the 0-back control conditions for subjects scanned on the two different magnets at baseline showed only small differences in the posterior cingulate. As will be shown below, no differences were seen in brain regions responsive to estradiol treatment. Thus, differences between different magnets do not explain the data pattern described below.

The MRI procedures were as follows. All subjects received the following MR sequences as part of the imaging protocol: (1) A sagittal T1-weighted spoiled gradient volumetric sequence oriented perpendicular to the anterior commissure (AC)-posterior commissure (PC) line using a repetition time (TR) of 9.9 ms, echo time (TE) of 4.6 ms, a flip angle of 8 degrees, number signal averages (NSA) 1.0, a field of view (FOV) of 256 mm, a 256 X 256 matrix, and 1.0 mm slice thickness with no gap for 140 contiguous slices. (2) An axial T2-weighted gradient spin echo (GRASE) sequence using the AC-PC line for slice positioning. Twenty eight contiguous slices of 5 mm slice thickness and no gap were acquired using TR 2466 ms, TE 80 ms, NSA 3.0 and FOV of 230 mm. All images were reviewed by a board-certified neuroradiologist to exclude intracranial pathology. fMRI was performed using EpiBOLD (echoplanar blood oxygenation level dependent) imaging. For the fMRI sequences, a single-shot, gradient-echo, echoplanar pulse sequence was used (TR 2500 ms/TE 35 ms/flip angle 90 degrees/1 NSA). Resolution was 2.5 mm × 2.8 mm × 5.0 mm. Thirty contiguous slices of 5mm thickness with no gap were obtained in the axial oblique plane, parallel to the AC-PC line using a FOV of 240 mm and a matrix size of 128 × 96. Field map correction for magnetic inhomogeneities was accomplished by acquiring images with offset TE at the end of the functional series.

Preprocessing and random effects analyses of the functional data were performed with Brain Voyager QX software (Brain Innovation, Maastricht, The Netherlands). Before the analyses were completed the following preprocessing steps were performed. Three-dimensional motion

correction to correct for small head movements was completed by alignment of all volumes to the first volume. Estimated translation and rotation movements never exceed 2 mm for any subject in these analyses. Further data preprocessing comprised of linear trend removal and filters for spatial (4 mm full-width half-maximum isotropic Gaussian kernel) as well as temporal (high pass filter: 1 cycle/run) smoothing to remove aliased signal correlated with background respiration and heart rate. Anatomical and functional images were co-registered and normalized to Talairach space. Statistical analysis was performed by multiple linear regression of the signal time course in each voxel. The expected BOLD signal change for each condition within a run was modeled by a canonical hemodynamic response function.

fMRI Analyses

fMRI analyses involved deriving one mean image per individual for each relevant contrast in the activation task by subtracting the baseline scan from the post-treatment scan (e.g., Post-treatment – Baseline) after accounting for the hemodynamic response function. These contrast images were further analyzed to examine the effects of estradiol compared to placebo treatment using standard independent samples *t*-test procedures in Brain Voyager. To correct for multiple comparisons, we used the cluster-level statistical threshold estimator from Brain Voyager QX to estimate a minimum cluster size threshold based on the approach of Forman, Cohen, Fitzgerald, Eddy, Mintun, and Noll (2005). The starting *p*-value used in this procedure was $p < 0.005$. This procedure estimated a minimum cluster size of 6 at an alpha level of 0.01.

Results

Activation Data

Differences in activation were examined between the estradiol and placebo treatment groups after controlling for baseline activation at each of the working memory load conditions. The N-back task typically activates a pattern of bifrontal, biparietal, and bicerebellar brain regions with increasing activation at increasing working memory loads (Braver et al. 1997). We examined alterations in this activation in the estradiol condition compared to the placebo condition (see Table 2). First, we examined potential differences between the treatment groups at baseline. No differences were found for the 2-, 1-, or 0-back conditions between treatment groups. We did find differences in the 3-back condition in the right insula and the left precentral gyrus. These regions are not usually activated in the N-back task. However, in an effort to control for these group differences we first controlled for baseline performance before examining the effects of three months of estrogen treatment.

We examined estradiol treatment effects in the 3-back condition after controlling for baseline performance (See Figure 1A). Increased activity was seen during estradiol treatment compared to placebo treatment in left frontal regions including the left superior frontal gyrus (BA 9) and left and right middle frontal gyrus (BA 10). The only posterior area of increased activation was in the precuneus (BA 31). In the 2-back condition there was similar increased activation for the estradiol compared to the placebo groups in BA 8 and 9 in the frontal lobe but this activation was in the right frontal lobe (see Figure 1B). There was no posterior activation in the 2-back condition. In the 1-back condition there were no areas of activation that were greater for the estrogen group. However greater activation for the placebo group was found in the right insula (BA 13) and the left precentral gyrus (BA 6). Finally, in the 0-back condition, there was greater activation in the estradiol group compared to the placebo group in the right superior temporal gyrus (BA 22 and BA 41).

Performance data

N-back performance data are presented as measures of sensitivity (d') and bias (C) (Snodgrass and Corwin 1988) as the percent correct performance for each working memory condition, 0-,

1-, 2-, and 3-back (see Table 3). Data were analyzed with a 2 (treatment: E2 vs. PLC) X 2 (test time: baseline vs. post-treatment) X 4 (working memory load: 0, 1, 2, 3) mixed model ANOVA.

Sensitivity as measured by d' is a measure of how different two classes of items are represented in standard deviation units. In the N-back task, the two classes of items are matches and mismatches for each N-back condition and larger numbers indicate greater sensitivity and greater accuracy. In the ANOVA for the sensitivity measure, the effect of working memory load was significant, $F(3,35) = 36.42, p < .001$. As Table 3 shows, participants performed the best on the 0-back condition; performance on the 1-back condition showed lower sensitivity than the 0-back ($t(39) = 3.63, p < .001$) and greater sensitivity than the 2- and 3- back conditions ($t(38) = 5.15, p < .001$ and $t(38) = 3.90, p < .001$, respectively). Performance on the 2- and 3-back conditions was not significantly different ($t(38) = 1.05, p > .29$).

Bias as measured by C represents the tendency for a subject to endorse a letter as a match or mismatch also represented in standard deviation units. Liberal response bias indicates that a subject calls a large number of responses matches in contrast to conservative bias indicating that the subject makes many mismatch responses. Bias scores of greater than 0 are conservative while bias scores less than 0 are liberal. For the bias measure (C) there was a 3-way interaction between test time, treatment, and working memory load, $F(3,105) = 3.31, p < .05$ (Table 3). To examine what was producing this interaction we examined bias at the baseline and post-treatment assessments independently. At baseline there was a treatment by working memory load interaction ($F(3,54) = 3.95, p < .05$) and a trend for a main effect of treatment ($F(1,18) = 3.48, p > .07$). The pattern of means showed that bias increased (became more conservative) for the placebo group across working memory load conditions at baseline. Bias for the estrogen group was more liberal compared to the placebo group and did not change across load at baseline. However, these group differences were observed at baseline before treatment was administered. At the post-treatment assessment, there was also an interaction of treatment and working memory load ($F(3,51) = 3.29, p < .05$) as well as a main effect of working memory load ($F(3,51) = 2.89, p < .05$). The pattern of means reveals a variable pattern of bias across working memory load for the treatment groups. On the 0- and 3-back conditions the estrogen group was more liberal compared to the placebo group and all subjects were more conservative overall as working memory load increased. Overall, there appears to be a group difference in the response bias that existed at baseline and this effect was variable across working memory load and estradiol treatment.

The Relationship Between Activation and Performance Data

Finally, we examined the relationship between the performance measures of d' and C and brain activation for the contrasts of interest described above. The main correlations of interest were the relationship of the performance measures after the three month treatment phase and average beta values for volumes of interest identified in the estradiol > placebo comparison after controlling for baseline for the 3-back and 2-back conditions described in Table 2. Medium-sized correlations were found between d' and activation in the left middle frontal gyrus (BA 8; $r = -.46, p < .05$), the left superior frontal gyrus (BA 10; $r = -.48, p < .05$) and the right precuneus (BA 31; $r = -.64, p < .003$). After correcting for multiple comparisons using an alpha level of .004 only the correlation between d' and the right precuneus remained significant. No correlations were found for activation and the bias measure C in the 3-back analysis. No significant correlations were found for either d' or C with any regions activated in the 2-back condition even at the more liberal .05 alpha level (largest $r = .27$, smallest $p > .26$). The direction of these relationships indicates that accuracy was decreased with increases in activation. The implications for these findings are discussed below.

Discussion

The present study is the first to examine the effects of estradiol treatment in postmenopausal women on working memory performance and related brain activation using a task that has a high manipulation component. The N-back sequential letter task allows for the parametric increase in working memory load, a key function in understanding the effects of medication interventions on task-related brain activity. The results partially supported our hypothesis that the estradiol group would show overall more activity in the frontal lobes. Three months of estradiol treatment increased frontal activation on the higher working memory load conditions (2-back and 3-back) of the N-back task compared to the placebo treatment group, but there was no estradiol effect on the lower working memory load conditions (0-back and 1-back). Additionally, there was no effect of estradiol treatment on the accuracy of N-back performance. These data support some prior findings in the literature on the effects of estradiol on working memory-related activation as well as provide new information about estradiol effects on brain activation in high working memory load conditions.

The activation data showed that estradiol effects were observed in the frontal regions in the more difficult conditions, 2- and 3-back. Frontal activation in the N-back task has been associated with active maintenance of information in working memory (Cohen et al. 1997; D'Esposito et al. 1999). The imaging data showed that with increasing working memory load, estradiol has a greater modulatory effect on the frontal functioning. Estradiol treatment affected brain activation predominantly in task conditions that were cognitively difficult. The 2- and 3-back conditions were difficult because of the need to update, maintain and evaluate the contents of information in working memory. As the N increased, the ability to do these active manipulations increased in difficulty. This increase is illustrated in the main effect of working memory load on the sensitivity measure. This study is the first to show that estradiol treatment influences frontal activation during these active manipulation conditions.

While estradiol effects were observed in the activation data, estradiol treatment did not affect the accuracy of working memory performance. Prior studies also found that estradiol increased frontal activation during a working memory task while there were also no effects of estradiol on task performance (Joffe et al. 2006; Shaywitz, et al. 1999; Smith et al. 2006). Performance may have been near ceiling in these prior studies making it difficult to evaluate what effects estradiol was having on related brain activation patterns. In the current study, performance was not at ceiling for the 2-back and 3-back conditions where the estradiol effects were observed on these high working memory load conditions. Thus, the use of a task that allowed for the parametric variation of working memory load allowed for the further specification of the effect of estradiol treatment on the cognitive processes supported by the frontal lobes.

To further explore the relationship between estradiol treatment, working memory performance and brain activation, we examined correlations between N-back performance and the significant areas of activation. The only correlation to survive the conservative Bonferroni correction for multiple comparisons was the negative relationship between right precuneus activity and the sensitivity measure for the 3-back analysis. The precuneus is activated during working memory tasks and has been implicated in a compensation response along with the frontal lobes seen when older adults are compared to younger adults (Rajah and McIntosh 2008). The other correlations also showed a negative relationship between the sensitivity measure and the frontal regions BA 8 and 10 such that increased activation in these areas was associated with decreased sensitivity and decreased accuracy. Thus, in the current study there is some indication that estradiol treatment and the related increased activation is not a compensatory response but rather indicates a decrease in working memory. Since the estradiol treatment did not directly affect working memory performance and only one correlation survived correction for multiple comparisons, caution is needed in the interpretation of these

data patterns. Prior studies have shown that increased frontal activation during a working memory task in older subjects is related to improved performance (e.g., Davis et al. 2008; Mattay et al. 2006). However, the effects of estradiol treatment on the relationship between frontal activation and performance may be different. Further studies with larger samples should investigate the possibility further that an increase in activation during estradiol treatment may not be beneficial to performance.

While this study is the first to systematically vary working memory load using a task that required constant updating and active maintenance of information in working memory, the results regarding the estradiol effects are similar to the prior literature using tasks more classically defined as short-term memory tasks where no manipulation or updating of information in working memory is required (Joffe et al. 2006; Shaywitz et al. 1999; Smith et al. 2006). However, the estrogens used in the current study and the three prior studies are all different. Shaywitz et al. (1999) used conjugated equine estrogen (CEE), Smith et al. (2006) used an ethinyl estradiol and norethindrone acetate preparation, and Joffe et al. (2006) used .05 mg/day of transdermal estradiol. Taken together these studies provide support for the proposal that the frontal lobes are particularly sensitive to estradiol modulation in postmenopausal women. Prior studies have found effects of estradiol on tasks thought of as hippocampally-supported such as verbal episodic memory (Maki and Resnick 2001). However, even episodic memory tasks have been shown to have frontal lobe components (e.g., Tulving et al. 1994). It is possible that estradiol effects on episodic memory tasks are modulated through frontal lobe effects. Future imaging studies should be designed to directly test this hypothesis.

There are some caveats to these data that should be considered. While this is a relatively large N placebo-controlled treatment study using fMRI, the estradiol treatment was a between subjects manipulation thus reducing our power to find small effects. Given the length of the hormone treatment, the intense challenge sessions, and the MRI scans, we decided to use a between subjects design so that subjects would be likely to complete the whole study. No subjects dropped out of the study once they started the hormone treatment. In an effort to control for group differences we scanned all women at baseline and controlled for baseline performance in order minimize any differences that may have been present between the groups after the random assignment to hormone condition. Finally, the lack of the estradiol effect on working memory performance was likely not a power issue that would have been solved with a few more subjects as the means across groups were almost equivalent.

In summary, these data support prior literature showing estrogen increased frontal lobe activity during working memory tasks, but suggest that the degree of this increase is related to cognitive load. Future studies should further investigate the cognitive processes subserved by the frontal lobes that are sensitive to estrogen as well as the relationship between the estrogen-related increase in activation and effects on cognitive performance.

Research Highlights

1. We examined the ability of estradiol to modulate the manipulation of information in working memory and related brain activation in postmenopausal women.
2. Twenty healthy postmenopausal women (mean age (SD) = 59.13 (5.5)) were randomly assigned to three months of 1 mg oral 17- β estradiol or placebo.
3. The fMRI data showed that women who were treated with estradiol for three months had increased frontal activation during the more difficult working memory load conditions compared to women treated with placebo.
4. This is the first study to show that estrogen-induced increases in brain activity were tied to cognitive load during a verbal working memory task.

5. These data suggest that estradiol treatment effects on cognition may be in part produced through modulation of frontal lobe functioning under difficult task conditions.

Acknowledgments

This work was supported by NIA K01 AG 030380, R01 AG021476, NCRR-00109, DoE SC 0001753. The authors wish to thank the research nursing staff of the University of Vermont GCRC for their hard work and support of this study and to our volunteers for their dedication to clinical research. The American Federation for Aging Research was a sponsor of this project.

References

- Baddeley, AD. Working Memory. Clarendon Press, Clarendon Press; 1986.
- Bopp KL, Verhaeghen P. Aging and verbal memory span: a meta-analysis. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences* 2005;60:P223–P233.
- Braver TS, Cohen JD, Nystrom LE, Jonides J, Smith EE, Noll DC. A Parametric Study of Prefrontal Cortex Involvement in Human Working Memory. *NeuroImage* 1997;5:49–62. [PubMed: 9038284]
- Cohen JD, Perlstein WM, Braver TS, Nystrom LE, Noll DC, Jonides J, Smith EE. Temporal dynamics of brain activation during a working memory task. *Nature* 1997;6625:604–608. [PubMed: 9121583]
- D’Esposito M, Postle BR, Ballard D, Lease J. Maintenance versus manipulation of information held in working memory: An event-related fMRI study. *Brain and Cognition* 1999;41:66–86. [PubMed: 10536086]
- Davis SW, Dennis NA, Daselaar SM, Fleck MS, Cabeza R. Que PASA? The posterior-anterior shift in aging. *Cereb Cortex* 2008;18:1201–9. [PubMed: 17925295]
- Duff SJ, Hampson E. A beneficial effect of estrogen on working memory in postmenopausal women taking hormone replacement therapy. *Hormones & Behavior* 2000;38:262–76. [PubMed: 11104644]
- Dumas, J.; Saykin, A.; McAllister, T.; McDonald, B.; Hynes, M.; West, J.; Newhouse, P. Muscarinic and nicotinic cholinergic modulation of verbal episodic memory encoding-induced activity in older women. 36th Annual Meeting, Society for Neuroscience; Atlanta, GA. 2006.
- Dumas JA, Hancur-Bucci C, Naylor M, Sites C, Newhouse P. Estrogen interacts with the cholinergic system to affect the verbal memory in postmenopausal women: evidence for the critical period hypothesis. *Hormones and Behavior* 2008a;53:159–169. [PubMed: 17964576]
- Dumas JA, Saykin AJ, McAllister TW, McDonald BC, Hynes ML, Newhouse PA. Nicotinic versus muscarinic blockade alters verbal working memory-related brain activity in older women. *American Journal of Geriatric Psychiatry* 2008b;16:272–282. [PubMed: 18378552]
- First, MBRLS.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for DSM-IV-TR Axis I Disorders. American Psychiatric Press Inc., American Psychiatric Press Inc; 2001. Patient Edition, SCID-I/P, 2/2001
- Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”: a practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research* 1975;12:189–198. [PubMed: 1202204]
- Joffe H, Hall JE, Gruber S, Sarmiento IA, Cohen LS, Yurgelun-Todd D, Martin KA. Estrogen therapy selectively enhances prefrontal cognitive processes: a randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. *Menopause* 2006;13:411–422. [PubMed: 16735938]
- Jurica, PJ.; Leitten, CL.; Mattis, S. Dementia Rating Scale-2. Psychological Assessment Resources Inc; Lutz, FL: 2001.
- Just MA, Carpenter PA. A capacity theory of comprehension: individual differences in working memory. *Psychological Review* 1992;99:122–149. [PubMed: 1546114]
- Keenan PA, Ezzat WH, Ginsburg K, Moore GJ. Prefrontal cortex as the site of estrogen’s affect on cognition. *Psychoneuroendocrinology* 2001;26:577–590. [PubMed: 11403979]

- Maki PM. A Systematic Review of Clinical Trials of Hormone Therapy on Cognitive Function: Effects of Age at Initiation and Progestin Use. *Annals of the New York Academy of Science* 2005;1052:182–197.
- Maki PM, Resnick S. Effects of estrogen on patterns of brain activity at rest and during cognitive activity: A review of neuroimaging studies. *NeuroImage* 2001;14:789–801. [PubMed: 11554798]
- Mattay VS, Fera F, Tessitore A, Hariri AR, Berman KF, Das S, Meyer-Lindenberg A, Goldberg TE, Callicott JH, Weinberger DR. Neurophysiological correlates of age-related changes in working memory capacity. *Neurosci Lett* 2006;392:32–7. [PubMed: 16213083]
- Rajah MN, McIntosh AR. Age-related differences in brain activity during verbal recency memory. *Brain Res* 2008;1199:111–25. [PubMed: 18282558]
- Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JE, Gass MLS, Stefanick ML, Lane DS, Hays J, Johnson KC, Coker LH, Dailey M, Bowen D. Effect of estrogen plus progestin on global cognitive function in postmenopausal women. *Journal of the American Medical Association* 2003;289:2663–2672. [PubMed: 12771113]
- Reisberg B, Ferris SH. Brief cognitive rating scale (BCRS). *Psychopharmacology Bulletin* 1988;24:629–635.
- Reisberg B, Ferris SH, de Leon MJ, Crook T. Global Deterioration Scale (GDS). *Psychopharmacology Bulletin* 1988;24:661–663. [PubMed: 3249768]
- Resnick SM, Coker LH, Maki PM, Rapp PR, Espeland MA, Shumaker SA. The Women's Health Initiative Study of Cognitive Aging (WHISCA): a randomized clinical trial of the effects of hormone therapy on age-associated cognitive decline. *Clinical Trials* 2004;1:440–450. [PubMed: 16279282]
- Shaywitz SE, Shaywitz BA, Pugh KR, Fulbright RK, Skudlarski P, Mencl WE, Constable RT, Naftolin F, Palter SF, Marchione KE, Katz L, Shankweiler DP, Fletcher JM, Lacadie C, Keltz M, Gore JC. Effect of estrogen on brain activation patterns in postmenopausal women during working memory tasks. *JAMA* 1999;281:1197–202. [PubMed: 10199429]
- Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, Fillit H, Stefanick ML, Hendrix SL, Lewis CE, Masaki K, Coker LH. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women. *Journal of the American Medical Association* 2004;291:2947–2957.
- Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN III, Assaf AR, Jackson RD, Kotchen JM, Wassertheil-Smoller S, Wactawski-Wende J. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women. *Journal of the American Medical Association* 2003;289:2651–2662. [PubMed: 12771112]
- Smith YR, Love T, Persad CC, Tkaczyk A, Nicols TE, Zubieta JK. Impact of combined estradiol and norethindrone therapy on visuospatial working memory assessed by functional magnetic resonance imaging. *Journal of Clinical Endocrinology & Metabolism* 2006;91:4476–4481. [PubMed: 16912129]
- Snodgrass J, Corwin J. Pragmatics of measuring recognition memory: applications to dementia and amnesia. *Journal of Experimental Psychology* 1988;117:34–50. [PubMed: 2966230]
- Tulving E, Kapur S, Craik FI, Moscovitch M, Houle S. Hemispheric encoding/retrieval asymmetry in episodic memory: positron emission tomography findings. *Proceedings of the National Academy of Science* 1994;91:2016–2020.
- Weber M, Mapstone M. Memory complaints and memory performance in the menopausal transition. *Menopause* 2009;16:1–7. [PubMed: 18971791]

A. 3-back

B. 2-back

Figure 1.

Activation map for estradiol treatment > placebo treatment after controlling for baseline performance ($p < .005$). Orange colors represent activation that is greater for the estradiol group relative to the placebo group. Blue colors represent activation that is greater for the placebo group relative to the estradiol group A. 3-back working memory load condition. B. 2-back working memory load condition. See text for results of statistical analysis

Table 1

Demographic data (means and standard deviations) for the estradiol and placebo treatment groups. No group differences were found in any of the demographic variables presented (smallest p : $p > .19$ for BMI).

	Treatment	
	Estradiol N=10	Placebo N=10
Age	59.1(6.1)	60.4(4.9)
BMI	26.9(4.2)	24.6(3.2)
Education	16.8(2.3)	15.8(2.2)
Years since menopause	11.3(6.1)	11.5(8.6)
Prior estrogen use	7/10	6/10
Years of prior estrogen use	7.0(7.4)	7.0(6.8)

Treatment comparisons at each working memory load, Talairach coordinates, cluster size, region descriptions (Brodmann's areas, BA), *t* values and uncorrected voxel-level *p* values.

Table 2

Contrast	Coordinates			Cluster Extent	Region Description	<i>t</i> value	<i>p</i> value
	x	y	z				
3 Back E2 > PLC	-19	31	39	176	Left middle frontal gyrus (BA 8)	3.26	0.0017
	-10	58	18	305	Left superior frontal gyrus (BA 10)	3.36	0.0013
	-10	46	36	150	Left superior frontal gyrus (BA 8)	3.27	0.0017
	23	25	36	108	Right middle frontal gyrus (BA 8)	3.32	0.0015
	17	19	-13	209	Right inferior frontal gyrus (BA 47)	3.44	0.0012
	47	-8	-9	443	Right middle temporal gyrus (BA 21)	3.50	0.0011
	-19	-14	42	126	Left cingulate gyrus (BA 24)	3.31	0.0015
	-16	-26	54	170	Left precentral gyrus (BA 4)	3.25	0.0018
	-13	-36	60	93	Left postcentral gyrus (BA 4)	3.32	0.0015
2 Back E2 > PLC	8	-68	24	235	Right precuneus (BA 31)	3.24	0.0018
	8	34	42	450	Right medial frontal gyrus (BA 8)	3.69	0.0006
	23	19	-15	305	Right inferior frontal gyrus (BA 47)	3.53	0.0008
	23	25	36	83	Right middle frontal gyrus (BA 8)	3.35	0.0011
	-13	-32	60	243	Left postcentral gyrus (BA 4)	3.64	0.0007
1 Back E2 > PLC	41	-20	19	274	Right insula (BA 13)	-3.40	0.0019
	-52	-2	18	249	Left precentral gyrus (BA 6)	-3.49	0.0009
0 Back E2 > PLC	50	-5	-9	308	Right middle temporal gyrus (BA 21)	3.47	0.0009
	44	-32	12	158	Right superior temporal gyrus (BA 41)	3.39	0.0011

Table 3

Sensitivity (d'), bias (C), and percent correct on the N-back task for the two treatment groups (E2 and PLC) for each time point across working memory load. Working memory load was the only significant effect ($ps < .001$) for d' and percent correct showing the 2- and 3- back conditions were more difficult than the 0- and 1-back condition. For C there was a three-way interaction between hormone treatment, test time and working memory load was significant ($p < .05$).

	Baseline		Three Months Treatment	
	PLC	E2	PLC	E2
0-back: d'	2.54 (.09)	2.46 (.09)	2.52 (.09)	2.50 (.09)
C	.20 (.05)	.30 (.05)	.03 (.05)	.18 (.05)
Percent correct	95.3 (1.7)	90.9 (1.7)	93.4 (1.6)	96.0 (1.5)
1-back: d'	2.12 (.19)	2.05 (.19)	2.15 (.20)	1.27 (.19)
C	.31 (.09)	.21 (.09)	.30 (.09)	.29 (.09)
Percent correct	83.3 (3.9)	83.8 (3.9)	83.5 (4.4)	85.0 (4.3)
2-back: d'	1.57 (.30)	1.25 (.30)	1.46 (.31)	1.27 (.30)
C	.30 (.10)	.08 (.10)	.16 (.10)	.34 (.10)
Percent correct	75.3 (4.3)	71.5 (4.2)	76.0 (6.0)	70.8 (5.9)
3-back: d'	1.47 (.21)	1.43 (.21)	1.93 (.22)	1.50 (.21)
C	.57 (.10)	.16 (.10)	.52 (.10)	.31 (.10)
Percent correct	73.5 (4.8)	72.8 (4.8)	80.0 (2.9)	76.1 (2.7)