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Estradiol treatment altered anticholinergic-related brain activation during working memory in postmenopausal women

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Abstract

Estradiol has been shown to affect cholinergic modulation of cognition in human and nonhuman animal models. This study examined the brain-based interaction of estradiol treatment and anticholinergic challenge in postmenopausal women during the performance of a working memory task and functional MRI. Twenty-four postmenopausal women were randomly and blindly placed on 1 mg oral 17- β estradiol or matching placebo pills for three months after which they participated in three anticholinergic challenge sessions. During the challenge sessions, subjects were administered the antimuscarinic drug scopolamine, the antinicotinic drug mecamylamine, or placebo. After drug administration, subjects completed a fMRI session during which time they performed a visual verbal N-back test of working memory. Results showed that scopolamine increased activation in the left medial frontal gyrus (BA 10) and mecamylamine increased activation in the left inferior frontal gyrus (BA 46). Estradiol treatment compared to placebo treatment significantly reduced the activation in this left medial frontal region during scopolamine challenge. Estradiol treatment also increased activation in the precuneus (BA 31) during mecamylamine challenge. These data are the first to show that estradiol modulated antimuscarinicand anitnicotinic-induced brain activity and suggest that estradiol affected cholinergic system regulation of cognition-related brain activation in humans.

Keywords

estradiol; cholinergic system; working memory; fMRI; postmenopausal women

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1. Introduction

Cognitive changes observed in healthy older adults include decreased attention, working memory, and episodic memory (e.g., (Verhaeghen et al., 1993). However, the relationship between cognitive aging and the underlying neurobiological changes is complex and remains an area of ongoing investigation. One neurotransmitter system shown to be important in attention and memory processes (Warburton and Rusted, 1993) and clearly relevant to aging (Bartus et al., 1982) is the cholinergic system. In addition, aging involves the loss of circulating estrogens for women. As the brain is an important target organ for the action of estrogen (Morrison et al., 2006), the loss of estrogen is hypothesized to play a role in the neurobiology of cognitive aging. Furthermore, the actions of the predominant circulating estrogen, estradiol on cholinergic functioning are a potential neurobiological mechanism to explain more broadly some of the effects of estradiol on brain functioning and cognition (Gibbs, 2010). This study examined the interaction between estradiol and the cholinergic system and the effects of this interaction on brain activation during a working memory task.

The withdrawal of estradiol after menopause has direct effects on brain regions involved in cognition as well as direct effects on the cholinergic system. Specifically, estrogen has been shown to modulate cholinergic neurotransmission in the brain (Gibbs, 1996, McMillan et al., 1996). Estrogen modulated cellular markers affecting cholinergic functioning in the hippocampus and the frontal lobes. For example, loss of estrogen after ovariectomy has been shown to decrease high affinity choline uptake (HACU), choline aetyltransferase (ChAT) activity, and ChAT mRNA levels (Luine et al., 1975, Luine et al., 1986, Singh et al., 1993, Gibbs, 1994). Estradiol treatment in ovarictomized rats was associated with increased choline uptake and ChAT activity in the frontal lobe and hippocampus (Simpkins et al., 1997). Estrogen therapy also had positive effects on cholinergic fiber patterns in a primate model with surgically menopausal monkeys. Cholinergic fiber density in the dorsolateral prefrontal cortex was decreased two years after ovariectomy relative to intact monkeys and this decrease was prevented if monkeys were treated with estrogen (Tinkler et al., 2004). Thus, estrogen treatment affected cholinergic circuits in the frontal lobe and hippocampus, a finding that is important for understanding age-related changes in attention and memory.

The effects of the estrogen-cholinergic interaction on behavior and cognition have been examined in human and nonhuman animal models (see Gibbs 2010 for a review). In rats, Packard (Packard, 1998) found that the memory-enhancing effects of estradiol injected directly into the hippocampus were blocked with the administration of the antimuscarinic drug scopolamine. Daniel and colleagues (Daniel et al., 2005) showed that estradiol did not enhance working memory performance on a Morris water maze task when a muscarinic M2 antagonist was injected directly into the hippocampus of ovariectomized rats. Conversely, in a nonhuman primate model the effects of scopolamine were reduced in ovariectomized animals treated with estrogen on a visuospatial cuing task of attention (Voytko, 2002). Thus, data from nonhuman animal models support the proposal that an intact cholinergic system is necessary to observe the beneficial effects of estrogen on cognition.

The estrogen-cholinergic interaction has also been examined in humans. Using an anticholinergic reversal model, Dumas and colleagues (Dumas et al., 2006) examined the effects of three months of 1 mg oral 17- β estradiol treatment compared to placebo on a battery of attention and memory tasks. We found that estradiol treatment reversed the impairment from antimuscarinic and antinicotinic medications on attention and speed tasks, specifically. A second study examined three months of 2 mg oral 17- β estradiol per day in the anticholinergic reversal model in younger compared to older postmenopausal women. Estradiol treatment reversed the impairment from anticholinergic drugs on a verbal memory

test for the younger postmenopausal women only (mean age 55 years) and estradiol appeared to increase impairments for older postmenopausal women (mean age 74 years; (Dumas et al., 2008a). A study in younger premenopausal women examined the effects of 31 days of 100 μ g/day transdermal estradiol in a scopolamine reversal model (Bartholomeusz et al., 2008). They found estradiol treatment improved spatial working memory performance but did not protect against the impairing effects of scopolamine.

Overall, these studies replicate and extend the findings seen in the nonhuman animal models described above suggesting that estradiol modulated cholinergic functioning to affect cognition. However, the specificity of the effects of the estradiol-cholinergic interaction on functional brain circuitry involved in attention and memory processes are unknown. Understanding the basis of this interaction is important for discerning the underlying neurobiological processes responsible for how estrogen modifies cholinergic function in relation to cognitive performance. The current study will provide a neurobiological index of this interaction as changes in task-related brain activation have been associated with both cholinergic drug manipulations (Dumas et al., 2008b, Dumas et al., 2010b), estrogen treatment after menopause (Shaywitz et al., 1999, Joffe et al., 2006, Smith et al., 2006, Dumas et al., 2010a), and normal aging (e.g., (Davis et al., 2008).

To assess how estradiol and cholinergic manipulations affected cognitive processes and related brain activation, the current study examined the effects of the estradiol-cholinergic interaction on functional brain activity during a working memory task in postmenopausal women. Recent data have shown in separate studies that cholinergic antagonists (Dumas et al., 2008b, Dumas et al., 2010b) and estradiol (Shaywitz et al., 1999, Joffe et al., 2006, Smith et al., 2006, Dumas et al., 2010a) modulated frontal lobe functioning during working memory in postmenopausal women. Depending on the task requirements Shaywitz et al. (1999) found that estrogen treatment increased frontal activation during retrieval and decreased parietal activation during encoding in working memory. Working memory is a cognitive process that involves the active maintenance and constant updating of a small amount of information held in memory over a short period of time (Baddeley (Baddeley, 1986, Just and Carpenter, 1992) and is impaired with increased age (e.g., (Verhaeghen et al., 1993). Working memory has been hypothesized to be a primary cognitive resource that supports a range of other higher cognitive functions (e.g., (Salthouse et al., 1989). The current study examined the neurobiological mechanisms involved in working memoryrelated brain activation in postmenopausal women by manipulating estradiol and the cholinergic system. More specifically, we used two medications that blocked both muscarinic and nicotinic receptor subtypes of the cholinergic system. Our prior studies showed similar activation patterns for muscarinic and nicotinic blockade effects on brain activation patterns, thus our hypotheses are the same for both receptor systems as detailed below.

Two hypotheses were tested in this study. First, we proposed that anti-cholinergic drugs would increase frontal lobe activation compared to placebo similar to our prior findings (Dumas et al., 2010b). With a temporary decrease in cholinergic functioning, healthy postmenopausal women would have increased activity in brain regions involved in task operations to maintain adequate performance. Our second hypothesis was that estradiol treatment would reverse the anticholinergic effects on brain activation, and women in the estradiol group would have reduced frontal activation compared to women in the placebo group after anticholinergic challenge. Our prior studies have shown that estradiol reversed the impairments observed after anticholinergic challenge on attention and memory tests (Dumas et al., 2006, Dumas et al., 2008a), thus we predict that similar reversal of the anticholinergic effects would be seen on brain activation patterns. An alternative hypothesis was that estradiol treatment would have effects that are independent of the cholinergic

manipulations and would result in nonspecific increases in frontal and parietal activation. Prior studies have also found that estrogen treatment increased frontal (Shaywitz et al., 1999, Joffe et al., 2006, Smith et al., 2006, Dumas et al., 2010a). and parietal (Shaywitz et al. 1999) activation during working memory in postmenopausal women. Thus, this study will provide evidence to evaluate the proposal that estradiol interacts with the cholinergic system to affect cognition-related brain activation.

2. Method

2.1 Participants

Participants were 25 cognitively normal postmenopausal women, ages 51-71 years, M(SD) = 59.1(5.5). Five additional participants passed the screening but withdrew before beginning hormone treatment because of the time commitment of the study. One subject had greater than 2 mm of motion on each study day on all scans during the MRI sessions and her data were excluded from the data analysis. Participants were randomly assigned to receive either three months of 1.0 mg oral 17- β estradiol (E2) per day or placebo. The participants who received estradiol were ages 51-71 years (M(SD) = 58.75(6.0), N = 12). The participants who received placebo were ages 51-67 years (M(SD) = 59.50(5.2), N = 12). There was no significant difference between the ages of these two groups (t(22) = .33, p = .74). See Table 1 for demographic characteristics.

Participants were recruited through notices and advertisements in local newspapers and direct mailings. Participants were required to be postmenopausal, without menses for one year and without surgically-induced menopause. Exclusion criteria included smoking, a history of breast cancer, and use of hormone therapy during the last year. Fifteen participants had previously taken hormone or estrogen therapy after menopause. The length of time of prior hormone use ranged from one week to 15 years and there were no differences between the two treatment groups (t(22) = .09; p = .93; See Table 1). Medical exclusion criteria for estradiol 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, carbamezepine, 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. Exclusion criteria for MRI scanning included claustrophobia, cardiac pace makers, other implanted metal devices, injuries to the eve involving metal, tattoos on the head or neck, and other moveable metal implants in the body. 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 participants were not alcohol dependent, and the caffeine criterion was used to ensure participants would not experience caffeine withdrawal on testing days.

Upon meeting these criteria, participants were approved for further screening at the University of Vermont (UVM) General Clinical Research Center (GCRC). After signing informed consent documents, participants provided a medical history, underwent a physical and laboratory tests assessing hematopoietic, renal, hepatic and hormonal function. Participants were cognitively evaluated using the Mini Mental State Exam (MMSE; (Folstein et al., 1975), Brief Cognitive Rating Scale (Reisberg and Ferris, 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). Participants were required to have an MMSE score greater than or equal to 27, a DRS score greater than or equal to 123, 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, participants completed the Beck Depression Inventory (BDI). A cut off score of 10 was used for the BDI, and participants scoring over this criterion were discontinued from further participation. All participants met these criteria for the cognitive and behavioral screening.

2.2 Estradiol Administration

After meeting all inclusion criteria, participants were randomly and blindly assigned to the estradiol or placebo condition for three months. In the estradiol condition, participants took 1 mg of oral 17- β estradiol per day for three months. In the placebo condition participants took similarly appearing placebo pills for three months. One of our previous studies showed that 1 mg of oral estradiol per day for three months is sufficient for observing a reversal of the effects of anticholinergic medications on cognitive performance (Dumas et al. 2006). After three months, participants completed three cholinergic challenge days (described below). During the time when participants were completing the challenge sessions that took approximately 3 weeks, participants continued taking their estradiol or placebo daily. After completion of the challenge days, all participants took 10 mg per day of medroxyprogesterone acetate for 12 days to produce sloughing of any endometrium that developed.

2.3 Challenge Procedure

After three months of estradiol or placebo treatment, participants came to the UVM GCRC for three cholinergic challenge days. Study days were at least 48 hours apart and were generally scheduled one week apart. On each challenge day, participants reported to the UVM GCRC by 0700. Each participant performed a baseline motor skill sobriety test to serve as a comparison to a second test before discharge in the afternoon. An intravenous line (IV) was inserted and blood was drawn for estradiol (E2) and estrone (E1) assays. A doubleblind, double placebo method of administration of the challenge drugs was followed. Participants received one of the following medications: 2.5 µg/kg scopolamine (SCOP) IV, 20 mg mecamylamine (MECA) orally, or placebo (IV and oral). At time 0, a pill was administered containing the MECA dose or placebo. Thirty minutes later, an injection of the SCOP dose or placebo was administered through the IV. On each day only one drug was active or both were placebo. We have used this dosing procedure in a number of prior studies and it ensured that the medications were active at the time when the cognitive testing during the MRI began (Dumas et al., 2006, Dumas et al., 2008a, Dumas et al., 2008b, Dumas et al., 2010b). The order of the drug administration across the three days was determined randomly but balanced for the two treatment groups. Two hours after oral pill administration and ninety minutes after the injection, the visual verbal N-back fMRI testing began at a running time of 120 minutes. After the fMRI session that took approximately 70 minutes, participants were given lunch. Vital signs and pupil diameter were assessed at six time points throughout the session. At the end of the study day, after passing the sobriety test to the satisfaction of the research nurse and covering physician, participants were discharged.

2.4 fMRI Working Memory Task

A visually presented verbal N-back sequential letter task was used to probe working memory circuitry, wherein participants 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. The 0-back control condition had a minimal working memory load; participants were asked to decide if the current letter matched a single target letter that was specified before the epoch 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. For the 3-back condition, participants were asked to make a match response if the current letter was the same as the letter three back. The 0-, 1-, 2-, and 3-back conditions were repeated three times in a counterbalanced order such that the same condition was not repeated two times in a row. In this block design task, participants responded to nine items in each block that took 27 seconds. A rest break followed with a plus sign (+) fixation for 12 seconds. The total time of the task was 8 minutes 12 seconds. Participants practiced the N-back task before drug dosing began on each challenge day to ensure they understood the task instructions.

Participants 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 programmed using the E-prime software package and presented by PC; the PC recorded subject responses and reaction times.

2.5 Behavioral Measures

At the beginning of each challenge day, participants completed the Profile of Mood States (POMS; (McNair et al., 1971), BDI and Beck Anxiety Inventory (BAI; (Beck and Steer, 1990) to obtain a baseline measure of mood before the testing procedures began. After the cognitive battery was completed, participants and the experimenters completed the following participant and observer behavioral assessment measures. Participants completed the POMS a second time as well as the Stanford Sleepiness Scale (Hoddes et al., 1973), Subjective Visual Analogue Scale (SVAS; (Newhouse et al., 1994), and a Physical Symptom Checklist (PSCL). The experimenter completed the Brief Psychiatric Rating Scale (BPRS; (Overall and Gorham, 1993) and Objective Visual Analogue Scale (OVAS; (Newhouse et al., 1994).

2.6 fMRI Scan Procedure and Preprocessing

For logistical reasons, the first 11 participants were scanned on one magnet while the last 14 participants 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 participants scanned on the two different magnets at baseline and collapsed across treatment group showed only small differences in the posterior cingulate. These differences did not survive correction for multiple comparisons. No differences were seen in this analysis in brain regions responsive to estradiol treatment and cholinergic challenge that are described below. Thus, differences between different magnets did not explain the data patterns described in the results section.

The MRI procedures were as follows. All participants 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 \times 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 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 boardcertified 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 for 197 volumes). Resolution was 2.5 mm \times 2.8 mm \times 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 \times 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 exceeded 2 mm for one participant on all study days and her data were replaced. All other participants met these movement criteria. Further data preprocessing included 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.

2.7 fMRI Analyses

Statistical analyses were performed using a random effects model. First, one mean image per individual for each relevant contrast in the N-back task was derived by subtracting the activation for the 0-back condition from the 3-back condition (e.g. 3-back – 0-back) after accounting for the hemodynamic response function. These contrast images were further analyzed to examine the effects of the cholinergic challenge drugs which was a within-subjects factor (e.g. SCOP – PLC; MECA – PLC) and the effects of estradiol treatment which was a between-subjects factor (e.g. E2 – PLC-TX) using standard ANOVA procedures in Brain Voyager.

In an effort 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 et al. (Forman et al., 1995). The starting *p*-value used in this procedure was p < 0.05. This procedure estimated a minimum cluster size of 31 voxels in functional space ($3 \times 3 \times 3$) or 793 mm clusters at an alpha level of 0.05 for each of the four analyses described below.

2.8 Working Memory Performance Analysis

Working memory performance on the N-back task was analyzed below using the signal detection measures of sensitivity (d') and bias (C; (Snodgrass and Corwin, 1988). Sensitivity is a measure of how different two classes of items are as measured by d' and is represented in standard deviation units. In the N-back task, the two classes of items are matches and mismatches for each of the working memory load conditions. Larger d's represent greater sensitivity and greater accuracy. Bias (C) is 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.

3. Results

First, we describe the patterns for the effects of muscarinic and nicotinic blockade on working memory performance. Then, we examine the ability of estradiol treatment to modify the activation produced by the anticholinergic challenges.

Activation patterns were examined for each of the working memory load conditions, 3-back, 2-back, and 1-back each compared to the 0-back control condition. When we compared each of the working memory load conditions to the 0-back condition, we observed the expected bilateral frontal, parietal and cerebellar activation (Braver et al., 1997, Cohen et al., 1997) during the N-back task. The goal of the current study was to examine the effects of cholinergic challenge and estradiol treatment on working memory-related brain activation. Overall, the patterns of activation were similar regarding the effects of challenge drugs and estrogen treatment for the 1-, 2-, and 3-back working memory load conditions. Below, we present only the data for the 3-back compared to the 0-back condition for brevity and comparability with our prior study (Dumas et al., 2008b).

3.1 Activation Data: Cholinergic Blockade Effects on Working Memory-Related Brain Activation

First, we examined activation patterns on the N-back sequential letter task for the placebo treatment group to establish the anticholinergic patterns of brain activation without the influence of estradiol treatment in the current sample. We examined brain activation for the placebo treatment group during the working memory task on the scopolamine challenge day compared to the placebo challenge day (see Figure 1a). Increased activation during scopolamine compared to placebo challenge was seen in the left medial frontal gyrus (BA 10 and BA 6), right inferior parietal lobule (BA 40), left cingulate gyrus (BA 23), right precuneus (BA 7), and right medial dorsal nucleus of the thalamus (see Table 2).

Next, we examined brain activation during working memory performance on the mecamylamine challenge day compared to the placebo challenge day (see Figure 1b). Increased activation for mecamylamine compared to placebo challenge was found in right inferior frontal gyrus (BA 46), right middle temporal gyrus (BA 21), and right superior temporal gyrus (BA 38; see Table 2).

3.2 Activation data: Estrogen Effects on Cholinergic Blockade

To examine the ability of estradiol to alter activation related to antimuscarinic challenge, we examined brain activation for both the estradiol and placebo treatment groups on the scopolamine challenge days. We found that the estradiol group had less activation compared to the placebo treatment group in the left medial frontal gyrus (BA 10), right anterior cingulate gyrus (BA 24), left inferior parietal lobule (BA 40), right insula (BA 13), and left superior temporal gyrus (BA 22; see Table 3 and Figure 2a).

Finally, when we examined brain activation for the estradiol and placebo treatment groups on the mecamylamine challenge day, a different pattern of results was observed (see Figure 2b). No difference was seen in frontal activation between treatment groups. Increased activation for the estradiol group compared to the placebo treatment group was seen in the right precuneus (BA 31) and right paracentral lobule (BA 5). Decreased activation for the estradiol group compared to the placebo treatment group was seen in the right parahippocampal gyrus (BA 34; see Table 3 and Figure 2b).

3.3 Working Memory Performance

To mirror the functional analyses we present the performance data on the 0- and 3-back conditions. Data were analyzed with a 2 (treatment: E2 versus PLC-TX) \times 3 (challenge: SCOP, MECA, PLC) \times 2 (working memory load: 0 versus 3) mixed model ANOVA for the d', percent correct, and C measures. Treatment was a between-subjects factor and challenge and working memory load were within-subjects factors.

The analysis of *d*' showed a main effect of working memory load (F(1,22) = 103.30, p < . 001; see Figure 3a). Performance on the 3-back condition was worse than performance on the 0-back condition. There was also an interaction of challenge and working memory load (F(2,43) = 5.70, p = .006). For the 0-back condition, scopolamine impaired performance relative to placebo (t(22) = 3.32, p = .003) and mecamylamine challenges (t(22) = 2.29, p = . 03). There was no difference between PLC and MECA (t(22) = 0.63, p = .53). There were no differences across challenge drugs for the 3-back condition (largest t(22) = 1.35, smallest p = .19). There were no main effects or interactions involving treatment group; however, there was a trend toward an interaction of treatment and working memory load (F(1,22) = 3.38, p = .08). The pattern of means showed that the treatment effect was larger for the 3-back condition with the estrogen group performing slightly worse than the placebo group. Overall the data patterns for the percent correct measure were the same as d' (See Figure 3b).

There was only a main effect of working memory load for C (F(1,22) = 10.99, p = .003) that showed that subjects were more liberal on the 3-back compared to the 0-back condition (see Figure 3c).

3.4 Correlations between Activation and Performance, and Hormone Levels

We examined correlations between the beta values from the significant clusters generated from the E2 – PLC-TX analysis for the scopolamine and mecamylamine challenges and the 3-back – 0-back subtraction for the d' measure. To mirror the imaging analyses and compute correlations between d' and the beta values from the activation clusters, we subtracted d' for 0-back from d' for 3-back. Because performance generally declined between the 0-back and 3-back conditions, the more negative the d' difference, the more performance declined in the 3-back relative to the 0-back condition. Thus, negative correlations indicated that as activation in a particular cluster increased there was a *greater* decline in performance from the 0- to the 3-back condition; positive correlations indicated that as activation increased there was a *smaller* decline in performance from the 0- to the 3-back condition.

On the scopolamine challenge day, we examined correlations between activation and performance for the placebo and estradiol treatment groups separately. For the placebo treatment group on the scopolamine challenge day, there was a negative correlation between activity in the right inferior parietal lobule (BA 40) and d' (r= –.62, p = .02). Greater activation in this region was associated with a greater decline in performance from the 0- to the 3-back condition. In contrast, for the estradiol treatment group on the scopolamine challenge day, there were no significant relationships between brain activation and performance. Thus, in the right inferior parietal lobule, a region known to be involved in N-back performance, estradiol treatment altered the negative relationship between activation and performance seen during placebo treatment.

On the mecamylamine challenge day there was only a significant negative correlation for the estradiol treatment group in the paracentral lobule (BA 5) and d' (r = -.64, p = .03), such that greater activation was associated with a greater decline in performance from the 0- to the 3-back condition. There were no correlations of performance and activation for the placebo treatment group during the mecamylamine challenge day. Thus, in the paracentral

lobule (BA 5), a region not often involved in working memory processes, estradiol treatment was associated with impaired 3-back relative to 0-back performance while this relationship was not observed in the placebo treatment group.

3.5 Correlations between Performance and Hormone Levels

To examine the relationship between blood hormone levels and performance, estradiol and estrone blood levels were obtained at the beginning of each challenge day. Estradiol treatment for three months produced greater circulating blood levels of estradiol (M(SD) = 67.10 (47.4) pg/ml) for the estradiol group than for the placebo group (7.67 (2.0) pg/ml); t(21) = 4.14, p < .001) and estrone (M(SD) = 312.20 (151.3) pg/ml) for the estradiol group than for the placebo group (M(SD) = 31.6(10.7) pg/ml; t(21) = 6.13, p < .001).

We examined correlations between blood hormone levels and performance on the N-back task for the E2 – PLC analyses on the scopolamine and mecamylamine challenge days separately. For the placebo treated group on the scopolamine challenge day there was a positive correlation between estradiol blood levels and performance (r=.70, p = .02). This data pattern suggested that higher estradiol levels were related to a smaller decline from the 0- to the 3-back condition. For the estradiol treated group on the scopolamine challenge day there was a negative correlation between estrone blood levels and performance (r=-.62, p = .03). Higher estrone levels in the estradiol treated group were related to a greater decline in performance from the 0- to the 3-back condition. Thus, lower blood levels of naturally circulating hormones in postmenopausal women in the placebo treatment group were related to *better* performance on the N-back task, while higher levels of hormones found in the estradiol treated group were related to a group were challenge.

For the placebo-treated group on the mecamylamine challenge day, there were negative correlations between performance and estradiol (r=.74, p = .01) and estrone (r=.75, p = .01) blood levels, suggesting that higher hormone levels were related to a greater decline in performance from the 0- to the 3-back condition. There were no relationships between performance and hormone levels for the estradiol treated group on the mecamylamine challenge day. These correlation patterns showed that on the mecamylamine challenge day levels of estradiol and estrone in the placebo group were associated with worse performance while in the estradiol treatment group there was no relationship between blood hormone levels and performance.

3.6 Behavioral Measures

Before beginning each challenge day, participants completed the POMS, BDI, and BAI questionnaires. To assess group differences after the three month treatment phase, we examined these measures on the first challenge day for all participants using an independent samples *t*-test. Overall, all scores on the POMS, BDI, and BAI were well below the clinical range for both groups. The two groups had equivalent mood ratings on five of the six subscales on the POMS as well as the total mood disturbance score. One difference existed for the Anger-Hostility measure (t(22) = 2.94, p = .01) that showed the estradiol group reported lower scores and less anger and hostility compared to the placebo group. In addition, the BAI showed differences between the groups (t(22) = 2.13, p = .04) with the estradiol group having lower scores and reporting less physical symptoms associated with anxiety. We did not have a baseline measure of anxiety before the treatment phase so we cannot conclude that the decreased anxiety, anger and hostility was related to estradiol treatment. No group differences were found for the BDI measure on the first challenge day.

Questionnaires assessing mood and physical symptoms were administered after the MRI when subjects returned to the GCRC to examine whether there were effects of estrogen

treatment and the challenge drugs on these measures. Overall, estradiol treatment influenced only a few measures and the data showed that estradiol treatment generally decreased negative mood. Additionally, the expected side effects from the anticholinergic medications were observed. These data patterns are the same as have been described in our prior studies and did not correlate with any of the brain activation patterns described above (Dumas et al., 2006, Dumas et al., 2008a, Dumas et al., 2008b).

3.7 Vital Signs

Blood pressure, pulse, and pupil diameter were monitored at six time points throughout the challenge day. Analyses were conducted on the maximum change score from the baseline measurement for each variable. Overall there were no main effects or interactions involving estrogen treatment on any of the vital signs measures. There were main effects of challenge similar to our prior studies (e.g., (Dumas et al., 2008a, Dumas et al., 2008b) that confirmed that our doses were sufficient to produce the expected central and peripheral effects.

4. Discussion

The current study was the first to examine the task-related functional brain circuitry associated with the estradiol-cholinergic interaction in postmenopausal women. The results showed that muscarinic and nicotinic blockade increased frontal lobe activity compared toplacebo challenge during working memory. Three months of estradiol treatment altered this increase in frontal activation and resulted in decreased frontal activation for the estradiol group compared to the placebo group after muscarinic blockade. Three months of estradiol also decreased parahippocampal activity and increased precuneus activation after nicotinic blockade. However, performance was on the working memory task was not affected by the cholinergic blockade or the estradiol treatment. The implications of the effects of the estradiol-cholinergic interaction on brain activation patterns and not on working memory performance are discussed below.

Estradiol treatment appeared to directly affect working memory-related brain circuitry that was sensitive to cholinergic modulation. We have previously shown in behavioral studies that estradiol treatment decreased impairments from anticholinergic challenges on attention and memory tasks (Dumas et al., 2006, Dumas et al., 2008a). The current data now demonstrate that the ability of estradiol to alter cholinergic-related brain activity associated with cognitive processing. While estradiol by itself has been shown to modulate working memory-related brain activation (e.g., (Joffe et al., 2006, Dumas et al., 2010a), it also clearly interacts with the cholinergic system-related cognitive processes.

The estradiol effect in this estradiol-cholinergic interaction study was specific to modifying activation associated with the cholinergic manipulations. Thus, consistent with the preclinical data described above, estradiol affected muscarinic- and nicotinic-related cognitive processes specifically. We have shown previously that estradiol treatment modulated the bilateral frontal activity for the larger working memory load conditions, 3-back and 2-back, such that there was greater activation for the estradiol group compared to the placebo group (Dumas et al., 2010a). The current data showed that scopolamine also increased activation in this same frontal area (BA 10) and mecamylamine modulated another frontal region known to be involved in working memory processing (BA 46). However, when we examined the effects of estradiol versus placebo treatment under scopolamine challenge the estradiol treatment modulated the antimuscarinic activation pattern with less activation relative to placebo. The effects of estradiol after nicotinic blockade were different such that there was decreased parahippocampal activity and increased precuneus activation.

The current data showed that anticholinergic blockade resulted in an "older" pattern of brain activation for postmenopausal women and estradiol treatment appeared to modify this pattern. Functional imaging studies examining age differences in activation during working memory tasks generally found that older adults showed increased frontal activation (e.g., (Cabeza et al., 2004) and decreased occipitotemporal activity (e.g., (Grady et al., 1994) relative to younger adults. While this activation pattern was first described by Grady et al. (1994), Davis and colleagues (Davis et al., 2008) labeled this pattern the posterior-anterior shift in aging (PASA).

We have proposed that age-related changes in cholinergic system functioning are responsible for the PASA pattern (Dumas and Newhouse, 2011). Prior fMRI studies have shown that anticholinergic drugs increased frontal activity (e.g., (Dumas et al., 2008b) while procholinergic drugs increased posterior activity (e.g., (Furey et al., 2000, Bentley et al., 2003); but see (Giessing et al., 2006) for an exception). The data from the current study showed that anticholinergic blockade caused increased frontal activation in our sample of postmenopausal women. Estradiol decreased this frontal activation during antimuscarinic challenge and increased posterior activation patterns that mimicked an "older" pattern of activity, estradiol treatment appeared to alter this pattern to one that is more consistent with the pattern observed in younger adults.

In the current study, the antimuscarinic and antinicotinic challenges affected different frontal brain regions. Moreover, estradiol treatment showed different cholinergic-related modulation patterns with each challenge drug as well as different patterns of correlations between activation, performance, and hormone levels. One explanation for these differences is that only one dose of each challenge drug was examined. The effects of these medications at different points in their dose response curves may be related to their effectiveness on working memory-related brain activation. Examining a full range of doses of both drugs may have resulted in alterations in both frontal and posterior areas and should be examined in future studies.

Although we observed the estradiol-cholinergic interaction on brain activation patterns, we did not observe this interaction on working memory performance. For the anticholinergic drugs there were small effects on N-back performance. However, we specifically used a lower dose of scopolamine in this study compared to our prior studies (Dumas et al., 2006, Dumas et al., 2008a) to reduce the risk of drowsiness during the fMRI session. There was only an effect of scopolamine on 0-back performance suggesting scopolamine was impairing attentional performance. However, there was no effect of anti-muscarinic blockade on the greater working memory load, probably as a result of the low dose of drug. There were also no effects of mecamylamine on working memory performance. In our prior studies (Dumas et al., 2006, Dumas et al., 2008a), the effects of nicotinic blockade were smaller than muscarinic blockade on cognitive performance and that is true for the current study as well. Thus, the differences in brain activation across challenge days and treatment groups are not an effect of differences in performance on the n-back task. Many prior studies have shown that performance impairment increased with increasing dose of both scopolamine (Sunderland et al., 1985, Newhouse et al., 1988) and mecamylamine (Newhouse et al., 1992, Newhouse et al., 1994). Thus, future studies using higher doses of the anticholinergic medications are likely to show performance impairments and the effects of these impairments on brain activation. Finally, there were no effects of estradiol treatment on working memory performance. Prior studies examining estrogen treatment and related working memory performance and brain functioning found no effects of estrogen treatment on working memory performance while effects on brain activation were observed (e.g. (Shaywitz et al., 1999, Joffe et al., 2006). Performance improvements with estrogen

treatment are inconsistent in healthy postmenopausal women (e.g., (Haskell et al., 1997, Maki et al., 2001).

A caveat that should be considered when interpreting the data in the current study is the relatively wide age range of the subjects in this study. We have previously found differential effects of estradiol treatment on the ability to reverse the cognitive impairment during anticholinergic challenge for younger postmenopausal women (mean age 55 years) compared to older postmenopausal women (mean age 74 years; (Dumas et al., 2008a). The distribution of ages across the age range in the present study (51-71 years) was not sufficient to do a similar kind of analysis using age as a grouping variable. We did not specifically recruit participants to obtain two different age groups of older and younger postmenopausal women. Future studies may specifically be designed to address this question but the current study was not powered to examine age effects of short-term estrogen treatment.

The cholinergic system is inarguably the most prominent neurochemical system involved in age- and disease-related deterioration in cognitive performance, especially in neurodegenerative dementias, e.g. Alzheimer's disease. We have shown that the cholinergic system is sensitive to hormonal state. Declining function or dysfunction of the basal forebrain cholinergic system may weaken attentional processing thereby increasing errors and distractibility (Hasselmo and Sarter, 2010). Such changes may be responsible in part for the perception of attentional impairment by perimenopausal and postmenopausal women (e.g., (Weber and Mapstone, 2009). However, further studies are needed to understand the mechanism in humans underlying estradiol's ability of modulate task-related brain activation, its interaction with cholinergic-related brain activation, and estradiol's role in the frontal-posterior shift in brain activation

We believe the current data inform the findings from the Women's Health Initiative (WHI) study including the Women's Health Initiative Memory Study (WHIMS) and the Women's Health Initiative Study of Cognitive Aging (WHISCA) that did not show positive effects of estrogen treatment on cognition (Coker et al., 2010). However, it should be noted that the estrogen used in the WHI study was conjugated equine estrogen (CEE) while we used $17-\beta$ estradiol in the current study. We have proposed previously that the benefits of estrogen on cognition require an intact cholinergic system (Dumas et al., 2008a). Because of extensive cognitive testing in the current study, we assume that women had intact cholinergic systems. To more fully test the hypothesis that estrogen has effects on cognition by modulating cholinergic functioning, it would be ideal to examine in future studies the estrogencholinergic interaction in women who have known impairments in cholinergic functioning like in mild cognitive impairment. The WHI may not have shown benefits on cognition because the women were older and perhaps had begun to experience negative changes in cholinergic system functioning, thus were past the ideal window for an estrogen benefit on cognition. Future studies should explicitly test the critical window hypothesis (Resnick and Henderson, 2002, Sherwin, 2005, Maki, 2006, Daniel and Bohacek, 2010, Gibbs, 2010) with regard to cholinergic system functioning by examining the relationship between neuronal or structural integrity, functional brain activity, and estrogen-related cognitive effects.

Overall, these data showed the ability of three months of estradiol treatment to modulate brain activation related to antimuscarinic and antinicotinic challenge in postmenopausal women. The anticholinergic drugs increased frontal activation during a working memory task and estradiol treatment reversed this frontal increase compared to placebo treatment. While a number of rodent (see (Gibbs, 2010) for a review) and human cognitive studies (Dumas et al., 2006, Dumas et al., 2008a) have shown the importance of the cholinergic system in observing effects of estradiol on cognition, the current study is the first to show that working memory-related brain regions are specifically modulated by the estradiol-

cholinergic interaction. These data have implications for models of cognitive aging and we propose a specific hypothesis regarding the role of the cholinergic system in producing agerelated patterns of brain activation that are modifiable by estrogen treatment.

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B.



Figure 1.

Activation map for scopolamine minus placebo challenge (Figure 1a) and mecamylamine minus placebo challenge (Figure 1b) for the 3-back minus 0-back conditions of the N-back task (p < .05). These data are for subjects in the placebo treatment group only. Orange colors represent activation that is greater for the scopolamine or mecamylamine day relative to the placebo challenge day. Blue colors represent activation that is greater for the placebo relative to either challenge drug.



B.



Figure 2.

Activation map for estradiol treatment minus placebo treatment for the 3-back minus 0-back conditions of the N-back task for subjects on the scopolamine challenge day (Figure 2a) and the mecamylamine challenge day (Figure 2b; p < .05). Orange colors represent activation that is greater for the estradiol treatment group relative to the placebo treatment group. Blue colors represent activation that is greater for the placebo treatment group relative to the estradiol treatment group.



Figure 3.

Sensitivity (*d*' Figure 3a), percent correct (Figure 3b), and bias (C, Figure 3c) with standard errors on the 0- and 3-back conditions for the two treatment groups (E2 and PLC-TX) on each challenge day (SCOP, MECA, PLC).

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 > .14 for BMI).

	Treat	tment
	Estradiol N=12	Placebo N=12
Age	58.75 (6.0)	59.5 (5.2)
BMI	26.48 (3.9)	24.29 (3.0)
Education	16.67 (2.3)	16 (2.1)
Years since menopause	11.29 (5.5)	10.8 (8.0)
Prior estrogen use	8/12	7/12
Years of prior estrogen use	6.67 (6.6)	7.01 (6.8)

Table 2

Effects of scopolamine and mecamylamine challenges compared to placebo challenge for subjects in the placebo treatment group including Talairach coordinates, cluster size, region descriptions (Brodmann's areas, BA), t values and uncorrected voxel-level p values.

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Contrast	Ŭ	ordinat	tes	Cluster Extent	Region Description	t value	<i>p</i> value
	x	y	z				
JLC-TX							
SCOP – PLC challenge							
	-13	53	9	1303	Left medial frontal gyrus (BA 10)	2.92	.0045
	-1	-16	48	1505	Left medial frontal gyrus (BA 6)	2.97	.0038
	53	-35	33	1198	Left inferior parietal lobule (BA 40)	3.14	.0022
	-4	-55	36	3603	Left cingulated gyrus (BA 23)	3.00	.0034
	5	-55	36	1747	Right precuneus (BA 7)	2.92	0043
	8	-18	٢	1052	Right medial dorsal nucleus of the thalamus	3.23	.0018
LC-TX							
MECA – PLC challenge							
	48	35	6	1018	Right inferior frontal gyrus (BA 46)	3.11	.0025
	61	-44	ю	1184	Right middle temporal gyrus (BA 21)	3.57	.0006
	38	8	-16	1171	Right superior temporal gyrus (BA 38)	3.12	.0025

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Table 3

Effects of estradiol treatment compared to placebo treatment for all subjects during the scopolamine and mecamylamine challenge days including Talairach coordinates, cluster size, region descriptions (Brodmann's areas, BA), t values and uncorrected voxel-level p values.

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1	1						
Contrast	ŭ	ordina	tes	Cluster Extent	Region Description	t value	<i>p</i> value
	x	y	z				
SCOP challenge							
E2 – PLC-TX	-12	50	9	2461	Left medial frontal gyrus (BA 10)	-3.20	.0019
	14	19	23	3335	Right anterior cingulate gyrus (BA 24)	-3.60	.0005
	-37	-37	36	1048	Left inferior parietal lobule (BA 40)	-4.01	.000
	48	-37	19	1324	Right insula (BA 13)	-3.37	.0011
	-44	-3	-9	1209	Left superior temporal gyrus (BA 22)	-3.20	.0019
MECA challenge							
E2 – PLC-TX	18	-65	26	3623	Right precuneus (BA 31)	3.30	.0014
	11	-33	44	066	Right paracentral lobule (BA 5)	2.98	.0037
	13	L	-13	907	Right parahippocampal gyrus (BA 34)	2.88	.0050