Cholinergic modulation of hippocampal activity during episodic memory encoding in postmenopausal women: a pilot study

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Abstract

Objective: The cholinergic system has been shown to modulate estrogen effects on cognitive performance in postmenopausal women. In an effort to further understand cholinergic contributions to cognition after menopause, this pilot study investigated the effects of two receptor-specific anticholinergic drugs on brain activation and episodic memory encoding in postmenopausal women not taking estrogen.

Methods: Six healthy postmenopausal women took part in three drug challenges using the antimuscarinic drug scopolamine (2.5 μg/kg IV), the antinicotinic drug mecamylamine (20 mg PO), and placebo. During functional magnetic resonance imaging, participants performed a visual-verbal continuous recognition memory test that allowed for the separation of encoding and recognition processes.

Results: Functional magnetic resonance imaging results showed greater hippocampal and frontal activation and less occipital activation during encoding relative to retrieval conditions. This pattern of activation was similar under both drug challenges.

Conclusions: These results suggest that the changes in the cholinergic system may, in part, be responsible for menopause-related increases in brain activation.

Key Words: Episodic memory – Cholinergic system – Postmenopausal women – Functional magnetic resonance imaging.

During and after menopause, women report changes in cognitive functioning.1 Episodic memory declines are one of the hallmark cognitive changes that occur after menopause and in normal aging.2,3 Changes in cholinergic system integrity have long been implicated as a neurobiological explanation for cognitive aging. For older women, cognitive aging may be compounded by the loss of circulating estrogen that occurs during menopause. Whereas neurotransmitter systems such as serotonin and dopamine have been associated with cognitive changes in aging, many studies have demonstrated the importance of the cholinergic system for observing estrogen effects on cognition (see Gibbs4 for a review). In an effort to understand the neurobiological changes that occur after menopause and their effects on cognition, we have previously examined the relationship between the cholinergic system and cognition in postmenopausal women.5,6 However, studies are only beginning to examine the effects of cholinergic modulation of brain activation and related cognitive performance in older adults, and only one study has examined these effects in postmenopausal women.7

The cholinergic system has been shown to be important for a number of cognitive processes in humans, including episodic memory. Prior studies have shown a relationship between changes in cholinergic functioning and impairment in episodic memory.8–11 In addition, studies of cholinergic challenges in younger subjects using the muscarinic cholinergic antagonist scopolamine (SCOP) reliably showed impairments in episodic memory free recall and recognition tests.12–14 However, in younger subjects, the antinicotinic drug mecamylamine (MECA) did not impair cognition on measures of attention and memory.15,16 Conversely, procholinergic drugs such as cholinesterase inhibitors produce modest improvements in episodic memory performance in younger adults.17 The effects of anticholinergic challenges on cognition have also been revealed by neuroimaging studies.18 Two studies examined the effects of SCOP challenge on cognition and related brain activation. Schon et al19 found that SCOP challenge decreased encoding-related activity in the right posterior parahippocampal and mid-fusiform gyri and in the

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hippocampal body in younger adults during a delayed matching to sample (DMTS) test of working memory. Sperling et al.20 examined the effects of SCOP challenge on brain activity during an associative learning task in younger men and also found an attenuation of learning-related activity in the fusiform gyrus, inferior prefrontal cortex, and hippocampus for SCOP relative to placebo challenges. However, these prior studies were in younger subjects. Dumas et al7 examined the effects of SCOP and MECA on brain activity related to working memory in a group of postmenopausal women. Placebo was compared relative to each drug, and similar effects for both drugs were found, with decreased activation in frontal brain areas during a working memory task.7

Two studies by Craig et al21,22 examined the effects of SCOP challenge on brain activation in young women after gonadotropin-releasing hormone agonist (GNRHa) treatment. The use of GNRHa acutely suppresses ovarian hormone production and is a model of acute menopause. Craig et al21 examined the combined effects of GNRHa treatment and SCOP challenge on recognition memory and found decreases in the left inferior frontal gyrus compared with the placebo challenge. In the second study, Craig et al22 examined working memory performance using a DMTS task in this model and found decreases in the bilateral frontal cortex and left parahippocampal gyrus after GNRHa treatment and SCOP challenge. These studies illustrate the importance of the relationship between estrogen status and cholinergic modulation of cognition and related brain activation.

Taken together, these studies are evidence for the proposal that brain regions involved in memory processing, such as the hippocampus and frontal lobe, are influenced by muscarinic modulation.7,18-22 Thus far, only Dumas et al7 have examined the effect of nicotinic blockade on brain activation in postmenopausal women on a working memory task. Further work is needed to characterize the effects of nicotinic blockade on brain activation patterns in other cognitive domains.

Thus far, most prior work on the effects of cholinergic modulation of cognition has been conducted in younger adults or mixed groups of older men and women; studies have only recently begun to examine the relationship between the functioning of the cholinergic system, estrogen loss, and subsequent therapy after menopause. Dumas et al5,6 have shown that a slight impairment in cholinergic functioning using a cholinergic antagonist challenge model is necessary to observe beneficial effects of estrogen on cognition. However, using a similar cholinergic challenge model, Bartholomeusz et al23 found no benefit of estrogen on cognition in younger premenopausal women. Thus, the relationship between estrogen and the cholinergic system remains to be further elucidated. The current pilot study was an attempt to understand the functioning of the cholinergic system in postmenopausal women in an effort to begin to examine the effects of modulation of this neurotransmitter system on cognition after menopause.

This study examined the effects of anticholinergic blockade on brain activation during a visual-verbal episodic memory task in postmenopausal women. We used a continuous recognition memory task to assess the effects of cholinergic blockade on both encoding- and retrieval-related brain activity. Prior studies using a continuous recognition paradigm (eg, Johnson et al24) have found increased hippocampal activity for new relative to old words in a sample of younger men and women. This finding has been interpreted as the hippocampus being more actively involved in detecting new items than in retrieval-related processes elicited by previously learned items.

The current study is part of a larger project that examined the effects of cholinergic antagonists on cognitive processes in postmenopausal women.7 Although prior research has shown that cholinergic systems have a greater role in encoding relative to retrieval,23 no study has thus far examined these processes using both pharmacological challenge and advanced neuroimaging techniques in postmenopausal women. The goal of this pilot study was to investigate the effects of anticholinergic challenge on related brain activation in a group of postmenopausal women. Based on the prior literature, we predicted decreases in encoding-related hippocampal activity after anticholinergic challenge.

METHODS

Participants

Participants were six cognitively normal women, aged 51 to 72 years (mean [SD] = 58.8 [9.1] y), who were postmenopausal for an average of 9.9 years (SD, 11.0 y) since their last menses. For consideration for this study, women were required to be postmenopausal, be without menses for 1 year, have follicle-stimulating hormone levels greater than 30 mIU/mL, have no surgically induced menopause, and not be using hormone therapy for at least 1 year. Only one woman had a prior history of estrogen use. She used estrogen for 6 months, and it was 6 years before study participation. These requirements for participation were used to ensure a homogeneous sample with regard to hormone status, which has been shown to affect brain activation.26 Exclusion criteria included having a history of breast cancer, being a smoker, being a heavy alcohol or coffee user, and having significant cardiovascular disease, asthma, active peptic ulcer, hypothyroidism, pyloric stenosis, narrow angle glaucoma, epilepsy, and a current or past axis I psychiatric disorder. Participants had an average of 16.6 years (SD, 1.4 y) of education.

Initial screening and study procedures took place at the University of Vermont (UVM) General Clinical Research Center. After signing informed consent documents, participants gave a medical history and underwent physical and laboratory tests assessing hematopoietic, renal, hepatic, and hormonal function. Participants were cognitively evaluated using the Mini-Mental State Examination,27 Brief Cognitive Rating Scale,28 and Mattis Dementia Rating Scale29 to establish a Global Deterioration Scale score rating degree of cognitive impairment.28 Participants were required to have a Mini-Mental State Examination score of 27 or higher, a Dementia Rating Scale score of 123 or higher, and a Global Deterioration Scale score of 1 or 2 for participation.
Behavioral screening consisted of a partial Structured Clinical Interview for DSM-IV-TR\(^\text{30}\) to rule out individuals with current or past axis I psychiatric disorder. Participants also completed the Beck Depression Inventory,\(^\text{31}\) with participants scoring higher than 10 being excluded from further participation. All participants met required criteria for the cognitive and behavioral screening.

**Cholinergic challenge procedure**

After screening at UVM, participants took part in three cholinergic challenge and functional magnetic resonance imaging (fMRI) sessions at Dartmouth-Hitchcock Medical Center. At each visit, participants performed a baseline motor skill sobriety test for comparison to a second test before discharge in the afternoon. An intravenous line was inserted and baseline vital signs were assessed. A double-blind, double-placebo method of administration of the challenge drugs was followed. Participants received one of the following medications: 2.5 \(\mu\)g/kg SCOP, 20 mg MECA, or placebo. SCOP was administered intravenously and MECA was administered orally. At time 0, a capsule was administered containing MECA or placebo. Thirty minutes later, an injection of SCOP or saline placebo was administered through the intravenous line. On each day, only one of the drugs was active or both were placebo. The order of the drug administration across the 3 days was counterbalanced. Ninety minutes after the injection and 2 hours after oral pill administration, the fMRI session began at a running time of 120 minutes. We have shown that 120 minutes after MECA administration and 90 minutes after SCOP administration are the optimal times for measuring the cognitive effects of these drugs.\(^\text{5-7}\)

Structural magnetic resonance imaging (MRI) and fMRI studies took approximately 70 minutes, after which participants and the experimenter completed behavioral assessment measures. In addition to the continuous recognition task described below, participants also completed an N-back test of working memory.\(^\text{7}\) After the MRI session, participants completed the Profile of Mood States,\(^\text{32}\) Stanford Sleepiness Scale,\(^\text{34}\) Subjective Visual Analogue Scale,\(^\text{34}\) and Physical Symptom Checklist. The experimenter completed the Brief Psychiatric Rating Scale\(^\text{35}\) and the Objective Visual Analogue Scale (OVAS).\(^\text{35}\)

Vital signs and pupil diameter were assessed at six time points throughout the session at running times of 0, 30, 60, 120, 210, and 240 minutes. 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.

**fMRI episodic memory task**

We used a visually presented verbal continuous recognition task in an event-related design to probe episodic memory-related brain areas. Participants saw single words appear for 2 seconds each. Words were selected to be high in their frequency of occurrence in English (mean [SD], 134.9 [84.4]) and were of medium concreteness (mean [SD], 4.0 [1.3]).\(^\text{37}\) Some sample words are market, command, and estate. Participants were instructed to indicate whether each word had been seen previously in the session. Participants saw a total of 20 words that appeared two times each, for a total of 40 items presented during each session. Participants made a response for each word to indicate whether it was a new word that had not been seen before or an old word that was a repeat from earlier on the list. Words were separated by an inter-stimulus interval that varied between 3 and 6 seconds. A different list of words was used for each study day.

Participants responded to all items by button press through an MRI-compatible fiber optic button response system (LUMItouch, Lightwave Medical Industries Ltd., Vancouver, BC, Canada) to indicate whether the item matched the target condition. Stimuli were delivered through a magnetic resonance-safe goggle and headphone system (Resonance Technology, Inc., Northridge, CA). Experimental tasks were presented by computer interface and were programmed using the Presentation software package; the computer recorded participant responses and reaction times.

**fMRI scan procedure and preprocessing**

All scans were acquired using the same GE Signa 1.5 Tesla Horizon LX scanner with echo speed gradients using a standard head radiofrequency coil. fMRI parameters were the following: repetition time, 2,500 milliseconds; echo time, 40 milliseconds; field of view, 24 cm; number of excitations (NEX) 1, yielding 29 contiguous 5-mm sagittal slices in a 64 \(\times\) 64 matrix with 3.75 mm\(^2\) in-plane resolution. Initial volumes before spin saturation were discarded. Spatial realignment was performed on all raw scan data before further analysis to remove any minor (subvoxel) motion-related signal change. All volumes for each participant were normalized into standardized Montreal Neurological Institute atlas space using SPM5 (Wellcome Department of Cognitive Neurology, University College, London, England). During spatial normalization, all scans were resampled to 2 mm\(^3\) isotropic voxels. Spatial smoothing to a full-width at half-maximum of 8 mm was performed before statistical analysis.

**fMRI analyses**

Whole brain and region of interest (ROI) fMRI analysis included statistical parametric mapping on a voxel-by-voxel basis using the general linear model approach\(^\text{38}\) as implemented in SPM5. This procedure involves deriving one mean image per individual for each relevant contrast in the activation task (eg, new > old words) after accounting for the hemodynamic response function. These contrast images were then used for the second-level multisubject random effects analyses\(^\text{39}\) using a one-way analysis of variance for drug factor (SCOP, MECA, and placebo). Given the preliminary nature of this study and the small sample size, the probability threshold was set at 0.01,uncorrected, with a minimum cluster extent \((k)\) of 50 contiguous voxels. For display purposes, activation maps are thresholded at \(P < 0.05\),uncorrected in the figures. These criteria were used in assessing predetermined search regions based on prior functional imaging and lesion studies, including medial temporal lobe (MTL) structures and prefrontal and occipital cortices. ROI analyses were conducted using the Wake Forest University PickAtlas\(^\text{40,41}\) to restrict...
TABLE 1. Sensitivity (d’ and bias (C) measures (SDs) for the continuous recognition memory task on each challenge day (n = 6)

<table>
<thead>
<tr>
<th>Challenge drug</th>
<th>Recognition memory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>d’ = 2.71 (0.54)</td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>C = 0.55 (0.35)</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>d’ = 2.75 (0.67)</td>
</tr>
<tr>
<td></td>
<td>C = 0.62 (0.46)</td>
</tr>
<tr>
<td></td>
<td>d’ = 2.45 (0.66)</td>
</tr>
<tr>
<td></td>
<td>C = 0.28 (0.51)</td>
</tr>
</tbody>
</table>

the search region to the hippocampi bilaterally. For this restricted search region, the probability threshold was set at 0.01 uncorrected at k = 20.

RESULTS

Performance data
Measures of sensitivity (d’) and bias (C) were used to assess recognition memory performance (Table 1). Sensitivity and bias are commonly used measures in studies of recognition memory (eg, Snodgrass and Corwin42). A measure of sensitivity indicates how different two classes of items are, represented in SD units. In this task, we compared old and new words. A measure of bias represents the tendency for a participant to endorse a word as old or new, also represented in SD units. Data were analyzed with a repeated-measures analysis of variance for the drug factor (SCOP, MECA, and placebo). The drug effect was not significant for d’; however, there was a trend (F2,10 = 3.31, P > 0.07): the pattern of means indicated that SCOP impaired performance relative to MECA and placebo challenges. There was no drug effect on C.

Imaging data
Memory encoding was examined by comparing brain activation patterns during processing of new versus old words (first vs second presentation). Data were analyzed with whole brain and ROI analyses were used to examine activity after SCOP and MECA challenges compared with the placebo challenge. Similar to our prior work,7 we examined the effect of anticholinergic challenge on brain activity by examining the drug/placebo contrasts in two different ways. In the placebo > drug comparisons, activation can be interpreted to represent areas that have decreased activity during anticholinergic challenge. In the drug > placebo comparisons, the activation may be related to an increase in compensatory processes that engage more brain areas during the drug challenge. These contrasts are described in the next section, with Montreal Neurological Institute coordinates, cluster extent, and region descriptions presented in Tables 2 and 3.

To examine the data in this small pilot study for outliers in this sample, we created scatter plots of standardized β values for each drug condition at each ROI described in Tables 2 and 3. We found that no participant had a β value that was more than 2 SDs from the mean in each drug condition in each ROI, indicating acceptable sample variability.

Whole brain analyses
First, we examined the placebo > SCOP and placebo > MECA comparisons to identify brain regions that showed less activity during cholinergic challenge (Table 2). In the placebo > SCOP comparison, significant clusters were apparent in the left occipital, temporal, and parietal lobes and right uncus. Several significant clusters were noted in the placebo > MECA comparison, including regions in the left parietal and occipital lobes as well as insula and MTL structures (parahippocampal gyrus and uncus). Significant clusters were also noted in the right occipital lobe, insula, and uncus. Although hippocampal regions did not show decreased activation during anticholinergic challenge as we hypothesized, greater encoding-related activation on placebo relative to drug was seen in other MTL structures.

TABLE 2. Placebo and drug contrasts, MNI coordinates, cluster extent, region descriptions (BA), uncorrected P value, and T value for whole brain analyses (N = 6)

<table>
<thead>
<tr>
<th>Contrast</th>
<th>MNI coordinates (X Y Z)</th>
<th>Cluster extent</th>
<th>Region description</th>
<th>Uncorrected P</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLC &gt; SCOP</td>
<td>−20 −92 28</td>
<td>169</td>
<td>Left occipital lobe, cuneus BA 19</td>
<td>0.01</td>
<td>4.77</td>
</tr>
<tr>
<td></td>
<td>−56 −48 −8</td>
<td>61</td>
<td>Left middle temporal gyrus BA 37</td>
<td>0.11</td>
<td>4.43</td>
</tr>
<tr>
<td></td>
<td>−14 −82 48</td>
<td>98</td>
<td>Left parietal lobe, precuneus BA 7</td>
<td>0.05</td>
<td>4.28</td>
</tr>
<tr>
<td></td>
<td>24 2 −30</td>
<td>145</td>
<td>Right uncus BA 28</td>
<td>0.02</td>
<td>3.57</td>
</tr>
<tr>
<td>PLC &gt; MECA</td>
<td>−22 −78 38</td>
<td>136</td>
<td>Left parietal lobe, precuneus BA 7</td>
<td>0.02</td>
<td>4.81</td>
</tr>
<tr>
<td></td>
<td>−26 −12 −30</td>
<td>64</td>
<td>Left parahippocampal gyrus BA 35</td>
<td>0.10</td>
<td>4.58</td>
</tr>
<tr>
<td></td>
<td>36 −8 −34</td>
<td>54</td>
<td>Right uncus BA 20</td>
<td>0.13</td>
<td>4.52</td>
</tr>
<tr>
<td></td>
<td>38 −88 16</td>
<td>73</td>
<td>Right middle occipital gyrus BA 19</td>
<td>0.08</td>
<td>4.25</td>
</tr>
<tr>
<td></td>
<td>−38 −68 −4</td>
<td>160</td>
<td>Left middle occipital gyrus BA 37</td>
<td>0.02</td>
<td>4.07</td>
</tr>
<tr>
<td></td>
<td>−50 −26 46</td>
<td>77</td>
<td>Left parietal lobe, postcentral gyrus BA 2</td>
<td>0.07</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>38 24 18</td>
<td>118</td>
<td>Right insula BA 13</td>
<td>0.03</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>−16 −104 18</td>
<td>100</td>
<td>Left occipital lobe, cuneus BA 18</td>
<td>0.05</td>
<td>3.62</td>
</tr>
<tr>
<td></td>
<td>−36 10 20</td>
<td>67</td>
<td>Left insula BA 13</td>
<td>0.09</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td>−22 −52 6</td>
<td>58</td>
<td>Left parahippocampal gyrus BA 30</td>
<td>0.12</td>
<td>3.55</td>
</tr>
<tr>
<td></td>
<td>−20 −46 −6</td>
<td>61</td>
<td>Left parahippocampal gyrus BA 19</td>
<td>0.11</td>
<td>3.01</td>
</tr>
<tr>
<td>SCOP &gt; PLC</td>
<td>−4 −48 32</td>
<td>51</td>
<td>Left cingulate gyrus BA 31</td>
<td>0.14</td>
<td>4.71</td>
</tr>
<tr>
<td></td>
<td>24 54 24</td>
<td>52</td>
<td>Right middle frontal gyrus BA 10</td>
<td>0.14</td>
<td>4.25</td>
</tr>
<tr>
<td></td>
<td>20 −10 18</td>
<td>109</td>
<td>Right thalamus</td>
<td>0.04</td>
<td>3.83</td>
</tr>
<tr>
<td></td>
<td>56 −62 −8</td>
<td>68</td>
<td>Right middle occipital gyrus BA 19</td>
<td>0.09</td>
<td>3.72</td>
</tr>
<tr>
<td>MECA &gt; PLC</td>
<td>−4 −46 32</td>
<td>70</td>
<td>Left cingulate gyrus BA 31</td>
<td>0.09</td>
<td>4.70</td>
</tr>
<tr>
<td></td>
<td>−2 −46 64</td>
<td>57</td>
<td>Left frontal lobe, paracentral lobule BA 5</td>
<td>0.12</td>
<td>4.18</td>
</tr>
<tr>
<td></td>
<td>28 −18 −12</td>
<td>61</td>
<td>Right hippocampus</td>
<td>0.11</td>
<td>4.15</td>
</tr>
<tr>
<td></td>
<td>20 −36 −4</td>
<td>421</td>
<td>Right hippocampus</td>
<td>&lt;0.01</td>
<td>4.07</td>
</tr>
</tbody>
</table>

MNI, Montreal Neurological Institute; BA, Brodmann’s area; PLC, placebo; SCOP, scopolamine; MECA, mecamylamine.
Next, we examined the SCOP > placebo and MECA > placebo contrasts to identify brain regions whose activity may be altered by the effects of the drugs during task performance (Table 2). In the SCOP > placebo comparison, significant clusters were apparent in the right frontal and occipital cortices and thalamus, as well as in the left cingulate gyrus. Significant clusters in the MECA > placebo contrast were noted in the left frontal lobe and cingulate gyrus and right hippocampus.

Examination of brain activation patterns displayed over the cortical surface (Fig. 1) shows similar overall patterns for encoding-related activity for both drug challenges. In the placebo relative to both drug challenges, there was increased left occipital activation. There were also similarities across both drug challenges compared with the placebo challenge with increased right prefrontal activation. These similar brain regions affected by both muscarinic and nicotinic blockade may represent areas impaired by cholinergic challenge (left occipital regions) and those recruited for task performance (right frontal regions) during cognitively difficult conditions of drug challenge.

**ROI analyses**

ROI analyses were conducted based on the hypothesis that less activation would be seen in the hippocampal region after anticholinergic drug challenge. We examined the comparisons of placebo > drug to detect the brain regions that had less activation during anticholinergic challenge. No significant clusters of activation were seen in the hippocampus for either the placebo SCOP or placebo MECA challenges. We also examined the reverse comparisons of drug > placebo to see if any regions showed increased activation during anticholinergic challenge. Both comparisons of SCOP > placebo and MECA > placebo showed increased activation in the right hippocampus. Thus, contrary to expectations, ROI analyses demonstrated increased right hippocampal activation in response to anticholinergic challenge (Fig. 2).

**Table 3.** Placebo and drug contrasts, MNI coordinates, cluster extent, region descriptions (Brodmann’s area), uncorrected P value, and T value for hippocampal ROI analyses (N = 6)

<table>
<thead>
<tr>
<th>Contrast</th>
<th>MNI coordinates (X Y Z)</th>
<th>Cluster extent</th>
<th>Region description</th>
<th>Uncorrected P</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLC &gt; SCOP</td>
<td></td>
<td></td>
<td>No significant clusters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLC &gt; MECA</td>
<td></td>
<td></td>
<td>No significant clusters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCOP &gt; PLC</td>
<td>28 −16 −12</td>
<td>21</td>
<td>Right hippocampus</td>
<td>0.34</td>
<td>3.66</td>
</tr>
<tr>
<td>MECA &gt; PLC</td>
<td>28 −18 −12</td>
<td>37</td>
<td>Right hippocampus</td>
<td>0.20</td>
<td>4.15</td>
</tr>
<tr>
<td></td>
<td>20 −36 4</td>
<td>45</td>
<td>Right hippocampus</td>
<td>0.16</td>
<td>4.07</td>
</tr>
</tbody>
</table>

MNI, Montreal Neurological Institute; ROI, region of interest; PLC, placebo; SCOP, scopolamine; MECA, mecamylamine.

FIG. 1. Surface rendering of activation maps for PLC > drug and drug > PLC comparisons (P < .05 for display purposes, k = 50). Note greater right frontal activation on both drugs relative to PLC and greater left occipital activation on PLC relative to both drugs. See text for the results of the statistical analyses. PLC, placebo; MECA, mecamylamine; SCOP, scopolamine.
Behavioral and vital signs data

As reported in our prior study, participants and the experimenter completed subjective rating forms after the scanning session. The behavioral data were analyzed separately for the SCOP and MECA challenges relative to the placebo challenge. Overall, there were no effects of either SCOP or MECA relative to placebo on the subjective measures: the Profile of Mood States, the Stanford Sleepiness Scale, the Subjective Visual Analogue Scale, the Brief Psychiatric Rating Scale, and the Physical Symptom Checklist. On the experimenter-completed OVAS, the expected effects of SCOP relative to placebo were observed, with participants being rated as more drowsy ($t_5 = 3.79$, $P < 0.05$), more fatigued ($t_5 = 2.61$, $P < 0.05$), and less alert ($t_5 = 2.60$, $P < 0.05$). There were no effects of MECA relative to placebo observed on the OVAS.

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. Only two significant changes were observed, both of which were expected. SCOP was associated with a higher pulse rate relative to placebo ($t_5 = 4.05$, $P < 0.01$). MECA was associated with a significantly greater decline in systolic blood pressure relative to placebo ($t_5 = 3.21$, $P < 0.05$).

DISCUSSION

This pilot study examined the effects of cholinergic blockade on episodic memory encoding and related brain activity in postmenopausal women. Prior studies have shown that cholinergic blockade impairs episodic memory performance in older adults. However, the effects of cholinergic blockade on brain activity related to specific components of episodic memory in postmenopausal women have not yet been investigated. This study found both specific increases and decreases in fMRI activation of task-relevant brain areas after anticholinergic challenge. These results are interpreted in terms of the role of the cholinergic system in episodic memory in postmenopausal women not taking estrogen.

Overall, the fMRI results were similar across muscarinic and nicotinic challenges. Both challenge drugs relative to placebo activated regions of the right hippocampus and right prefrontal cortex. Anticholinergic drugs, compared with placebo, also were associated with reduced activity in the occipital lobe. Although this is a pilot study with a small number of participants, the pattern of results leads to interesting hypotheses about the role of the cholinergic system in brain changes in cognition in postmenopausal women that can be investigated in larger studies.

On the basis of two prior studies examining the effects of scopolamine on brain activation, we predicted a decrease in hippocampal activity after the SCOP challenge. However, our results showed increases in right hippocampal activity for both SCOP and MECA relative to placebo. This data pattern is the opposite of what was found in prior SCOP challenge studies. However, there are two important differences between the designs of these studies. First, Sperling et al tested 10 young men aged 29 to 35 years and Schon et al tested 30 men and women with an average age of 22 years. Age effects in cholinergic challenges have been shown previously (eg, Newhouse et al and Sunderland et al). Although there are no fMRI challenge studies with a direct comparison between older and younger adults as well as men and women, it is probable that the magnitude and directionality of the cholinergic responsiveness of younger men and women and...
postmenopausal women are not the same. Second, the tasks are substantially different: memory for words in the current study, a face-name memory paradigm in Sperling et al.20 and a DMTS task in Schon et al.19 In addition, different task comparisons were used in each study. We examined activation for new versus old words. Sperling et al20 described the drug effects from the face-name pairs versus fixation comparison, and Schon et al19 examined delay activation between the study and test in the DMTS task. Thus, because of participant and task differences, these studies are not directly comparable although they each used a SCOP challenge.

The present data replicated prior studies examining the detection of new items in the context of a continuous recognition task.24 This study found increases in hippocampal activity for new words relative to old words. This pattern has been interpreted as implicating a role for the hippocampus in novelty detection. In the current study, in the context of cholinergic challenge, the new > old effect was increased further in the drug conditions. The challenge conditions may thus shift attentional resources to focus more on novel information at the expense of detecting the repetition of the old item. Increased hippocampal activity caused by anticholinergic drugs may also represent increased demand on hippocampal processes in the face of partial cholinergic blockade. This data pattern is complementary to studies examining cholinergic stimulation that have shown decreases in task-related activity.44,45

The present data also showed increased frontal activation in drug challenge conditions relative to placebo. This data pattern is different from the pattern found in the Craig et al21 study. The model of acute ovarian suppression may not be directly relevant to women who underwent natural menopause, as in the current study. In the current study, there were no statistically significant drug effects on performance, but there was a trend level effect of SCOP to impair performance relative to the placebo condition. The corresponding additional activation may be excess activity that does not assist in task performance. Perhaps, if the task was more difficult or the drug dose was greater, we would have observed significant performance effects of SCOP. In addition, performance after MECA was similar to that after placebo, but the activation after the MECA challenge was similar to that after the SCOP challenge. With the current sample size, it is difficult to tell whether or how performance correlated with activation. We hypothesize that the activation increase in the MECA condition may be compensatory in nature since performance was similar to placebo performance. Further work is needed to examine the relationship between performance and brain activation under different drug challenges.

In addition to the hippocampal activation patterns, there was also frontal and occipital activation that was sensitive to the drug challenge conditions. One hypothesis to further probe this data pattern is that the cholinergic system may be involved in creating the posterior anterior shift in aging (PASA) pattern.46 PASA has been described in studies of older and younger adults such that increases in frontal activity are observed for older adults and increases in occipital activity are observed for younger adults across measures of attention, working memory, and episodic memory. We hypothesize that the patterns observed under anticholinergic challenge may indicate a role for the cholinergic system in producing the PASA pattern. The anticholinergic challenge in this study resulted in increased frontal activity and decreased occipital activity, as is often seen in studies of older adults relative to younger adults. The addition of estrogen treatment to this model may allow for the assessment of the role of estrogen to modulate this aging-related pattern of activation resulting from temporary cholinergic impairment. Further studies are needed to test this hypothesis directly.

There are some caveats with this study that should be noted. This was a small exploratory study of postmenopausal women. These data patterns should be replicated with a larger sample. We have previously shown significant effects of estrogen on anticholinergic responsivity in older women.5,6 The next step is to examine the effects of estrogen treatment and its interaction with cholinergic challenge in postmenopausal women. This would allow us to make conclusions about whether there is a role for estrogen therapy in maintaining cholinergic functioning in older women.

In addition, we did not observe significant drug-related effects on performance. This continuous recognition memory task required the participants to make an old/new judgment. Generally, recognition memory tasks are considered easier than other measures of episodic memory such as free recall. Studies examining the role of the cholinergic system in cognition have shown that the cholinergic system is increasingly engaged to the extent that a task is difficult and requires effortful processing.25,47 In addition, we believe that because the drugs did not impair recognition performance yet still had effects on related brain activation, we are able to draw conclusions about the effects of the drugs on brain circuitry related to task performance in the face of cholinergic blockade, as the difference is not simply one of a performance difference between the placebo and drug conditions. Finally, because of the limited number of items used in this task, it was not possible to do a subsequent memory analysis. Future studies should design the task with more items so that performance on the memory task can be more tightly tied to the activation data.

**CONCLUSIONS**

Anticholinergic challenge during a continuous recognition task in postmenopausal women has preliminarily revealed a pattern of brain activation that displays the effects of temporary cholinergic impairment in a model with no circulating estrogen in postmenopausal women. These results provide the first step in describing cholinergic functioning and the relationship to cognition and related brain activation after menopause. A similar study design should be used to examine the effects of the subsequent treatment with estrogen in postmenopausal women in an effort to further elucidate the neurobiological and cognitive effects of estrogen treatment after menopause.
CHOLINERGIC BLOCKADE AND EPISODIC MEMORY

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