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Nicotinic Versus Muscarinic Blockade Alters Verbal Working Memory-Related Brain Activity in Older Women

Julie A. Dumas, Ph.D.¹, Andrew J. Saykin, Psy.D.^{2,3}, Thomas W. McAllister, M.D.³, Brenna C. McDonald, Psy.D.^{2,3}, Mary L. Hynes, R.N.³, and Paul A. Newhouse, M.D.^{1,*}

¹Clinical Neuroscience Research Unit, Department of Psychiatry, University of Vermont College of Medicine

²Center for Neuroimaging, Department of Radiology, Indiana University School of Medicine

³Brain Imaging Laboratory, Department of Psychiatry, Dartmouth Medical School

Abstract

Objectives—An important aspect of furthering our understanding of the CNS function after menopause is to examine the cerebral circuitry that appears to be influenced by cholinergic antagonist drugs in the presence and absence of estrogen. This pilot study investigated the effects of two anticholinergic drugs on brain activation and working memory performance in postmenopausal women not taking estrogen. This approach simulates the effects of age- or disease-related neuroreceptor and/or neuronal loss by temporarily blocking pre- and postsynaptic muscarinic and nicotinic cholinergic receptors.

Design—Six healthy postmenopausal women took part in three drug challenges using the anti-nicotinic drug mecamylamine (MECA, 20 mg, oral), the anti-muscarinic drug scopolamine (SCOP, 2.5 µg/kg, IV), and placebo during fMRI. The cognitive measure was a visually presented verbal N-back test of working memory.

Results—Neither MECA nor SCOP significantly impaired performance on the verbal N-back. Functional MRI results showed greater increases in frontal lobe activation in the placebo condition relative to each drug condition with different specific regional activation for MECA and SCOP.

Conclusions—These preliminary results suggest that brain activation patterns are sensitive to cholinergic modulation in postmenopausal women and that differential effects may be observed following nicotinic versus muscarinic blockade. This approach offers a potentially valuable method for modeling age-related changes in brain function, and the findings may have implications for cholinergic contributions to normal and pathologic aging.

Keywords

working memory; postmenopausal women; fMRI; cholinergic system

Objective

Converging evidence from psychopharmacological, neuroimaging, and psychological studies shows that the cholinergic system has a specific modulatory role in cognitive processing. In humans, the cholinergic system has been implicated in many aspects of

*Address for Correspondence (PN): Clinical Neuroscience Research Unit, Department of Psychiatry, University of Vermont College of Medicine, 1 South Prospect St., Burlington, VT 05401, Voice: (802) 847-4560, Fax: (802) 847-7889, Paul.Newhouse@uvm.edu.

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cognition including the partitioning of attentional resources, working memory, inhibition of irrelevant information, and improved performance on effortful tasks (1). Prior research has shown that older adults perform more poorly than younger adults in a variety of cognitive domains including working memory (2). Cholinergic deficits due to degeneration of basal forebrain cholinergic nuclei appear to be intimately tied to the cognitive deficits in attention, learning, and memory in patients with neurodegenerative disorders such as Alzheimer's disease and Lewy body dementia (3). Further, the activity of the CNS cholinergic system may be a primary determinant of the effectiveness of attentional, learning, and memory mechanisms (4). Intriguingly, cognitive symptoms reported by women at menopause also include difficulties in memory, attention, and word-finding, and some studies have shown an acceleration of cognitive problems of aging after menopause (5). Women also have a higher risk than men of developing cholinergic-related dementing disorders such as Alzheimer's disease (6).

What has not yet been fully elucidated is how the effect of aging on neurotransmitter systems affects the cognitive processes these systems support. This study examined the effects of cholinergic antagonists on working memory performance and related brain activation in cognitively normal postmenopausal women without hormone therapy. Specifically, the effects of nicotinic and muscarinic blockade on working memory performance and brain activation patterns were examined in older women.

In the working memory models of Baddeley (7) and Norman and Shallice (8) the central executive or supervisory attention system is a limited capacity processing resource that coordinates necessary operations for a task. Warburton and Rusted (9) proposed that the cholinergic system modulates processes that are supported by a limited capacity central executive and influences information processing during tasks that engage the control processes for the allocation of cognitive resources. Additionally, age-related changes in cognition may occur as a result of impairments in allocating necessary resources to a task, thereby implicating negative alterations in the cholinergic system as one of the main factors in cognitive aging. Thus, there is a link between cholinergic dysfunction and age-related cognitive dysfunction.

Prior research by Sunderland and Newhouse using scopolamine (SCOP), a muscarinic antagonist, and mecamlamine (MECA), a nicotinic antagonist, have shown that these agents can be used to simulate age and disease-related cognitive impairments (10-14). These studies have shown that muscarinic and nicotinic blockade impairs performance on initial sensory processing, attention, and psychomotor function, thereby implicating a role for the cholinergic system in the initial processing and encoding of information into memory. Thus age- and disease-related differences in cholinergic system integrity may influence sensitivity to anti-nicotinic challenge on cognitive performance.

Actions of the cholinergic system in cognitive processing have also been revealed by neuroimaging studies (see (15) for a review). Increased cortical activity after physostigmine, a cholinesterase inhibitor, compared to placebo has been shown during a working memory task in extrastriate and intraparietal areas during encoding, but not during retrieval (16). Physostigmine administration also facilitated visual attention by increasing activity in the extrastriate cortex during a repetition priming task (17). A number of studies have examined the effect of SCOP on brain activation during cognitive task performance in younger adults (18-22) and in older adults (23). In general, when SCOP impaired performance, there was also a decrease in brain activity relative to placebo in regions required for task performance. The tasks utilized in these studies were auditory conditioning (19), repetition priming (20), associative encoding (18), delayed-matching-to-sample (21), recognition memory (22), and object location learning (23). Nicotinic blockade with MECA has not been examined in

humans using fMRI, nor have the effects of nicotinic or muscarinic blockade on working memory.

The current study further examined the activity associated with working memory and interaction with the cholinergic system after menopause. A prior study by Green et al. (24) showed that cholinergic blockade can impair performance on the N-back test of working memory in younger adults. The current study examined the effects of nicotinic and muscarinic cholinergic blockade separately on N-back performance and associated brain activation in cognitively normal older postmenopausal women utilizing fMRI. Given the proposed role of the cholinergic system in central executive function and the known age-related changes in working memory, we utilized an anticholinergic challenge paradigm to examine the effects of mecamylamine and scopolamine on working memory performance and brain activation in older postmenopausal women. We hypothesized that anticholinergic drugs would result in decreases in frontal cortical activation relative to placebo during a working memory task.

Method

Subjects

Subjects were 6 cognitively normal women, ages 51-72, $M = 58.8$ ($SD = 9.1$) who were postmenopausal for an average of 9.9 years since their last menses ($SD = 11.0$). Subjects had an average of 16.6 years of education ($SD = 1.4$). Subjects were required to be postmenopausal, without menses for one year, have FSH levels > 30 mIU/ml, without surgically induced menopause, and without use of hormone therapy for at least one year. These requirements for participation were utilized to ensure a homogeneous sample with regard to hormone status which has been shown to affect brain activation (i.e. (25)). Exclusion criteria included history of breast cancer, smoking, heavy alcohol or coffee use, significant cardiovascular disease, asthma, active peptic ulcer, hyperthyroidism, pyloric stenosis, narrow angle glaucoma, epilepsy, or current or past Axis I psychiatric disorder.

Initial screening and study procedures took place at the University of Vermont (UVM) General Clinical Research Center (GCRC). After signing informed consent documents, subjects gave a medical history and underwent a physical and laboratory tests assessing hematopoietic, renal, hepatic and hormonal function. Subjects were cognitively evaluated using the Mini Mental State Exam (MMSE; (26)), Brief Cognitive Rating Scale (27), and the Mattis Dementia Rating Scale (DRS, (28)) to establish a Global Deterioration Scale score (GDS) rating the degree of cognitive impairment (27). 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; (29)) to establish the presence/absence of Axis I psychiatric disorders. In addition, subjects completed the Beck Depression Inventory (BDI, (30)). 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 required criteria for the cognitive and behavioral screening.

Cholinergic Challenge Procedure

After screening at UVM, subjects took part in three cholinergic challenge and fMRI testing sessions at Dartmouth-Hitchcock Medical Center (DHMC). At each visit subjects performed a baseline motor skill sobriety test to have as a comparison to a second test before discharge in the afternoon. An intravenous line (IV) was inserted and baseline vitals were assessed. A double-blind, double placebo method of administration of the challenge drugs was followed. Subjects received one of the following medications: 2.5 μ 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 one of the SCOP doses or saline placebo was administered through the IV. On each day, only one of the drugs was active or both were placebo. The order of the drug administration across the three days was counterbalanced. Ninety minutes after the injection and two hours after oral pill administration, the fMRI session began at a running time of 120 minutes. Structural and functional MRI studies took approximately 70 minutes, after which subjects and the experimenter completed behavioral assessment measures. Subjects completed the Profile of Mood States (POMS; (31)), Stanford Sleepiness Scale (32), Subjective Visual Analogue Scale (SVAS; (14)), and a Physical Symptom Checklist (PSCL). The experimenter completed the Brief Psychiatric Rating Scale (BPRS; (33)) and Objective Visual Analogue Scale (OVAS; (14)).

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, subjects were discharged.

fMRI Working Memory Task

We used a visually presented verbal N-back task to probe working memory circuitry, wherein subjects saw a string of consonant letters (except L, W, and Y), presented in upper or lower case to control for pattern recognition, 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; subjects 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 one. 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 followed the same pattern. Subjects responded to all items by button press through an MRI compatible fiber optic button response system (LUMItouch, Lightwave Medical Industries Ltd., Vancouver, BC) to indicate whether the item matched the target condition. Stimuli were delivered through an MR-safe goggle and headphone system (Resonance Technology, Inc., Northridge, CA). Experimental tasks were presented by PC interface and were programmed using the Presentation software package; the PC recorded subject responses and reaction times. This task is highly robust in producing bilateral frontal and parietal activation in healthy young and elderly controls and is sensitive to differences in patient groups (34-38).

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 RF coil. fMRI parameters were TR 2500 ms, TE 40 ms, FOV 24 cm, NEX 1, yielding 29 contiguous 5 mm sagittal slices in a 64×64 matrix with 3.75 mm^2 in-plane resolution. Initial volumes prior to spin saturation were discarded. Spatial realignment was performed on all raw scan data prior to further analysis to remove any minor (subvoxel) motion-related signal change. All volumes for each subject were normalized into standardized Montreal Neurological Institute (MNI) atlas space using SPM2 (Wellcome Department of Cognitive Neurology, University College, London). During spatial normalization all scans were resampled to 2 mm^3 isotropic voxels. Spatial smoothing to a full width half maximum of 10 mm was performed prior to statistical analysis.

fMRI Analyses

fMRI analysis included statistical parametric mapping on a voxel-by-voxel basis using the general linear model approach (39) as implemented in SPM2. This procedure involves

deriving one mean image per individual for each relevant contrast in the activation task (e.g., 3>0 back) after accounting for the hemodynamic response function. These contrast images were then used for the second level multi-subject/between-group random effects analyses (40). These contrast images were further analyzed using standard paired t-test and ANOVA (analysis of variance) procedures in SPM2. Given the preliminary nature of this study and small sample size, the critical significance level for group-level analyses was based on clusters of activated voxels with the probability threshold set at $p_{corr} < 0.05$ and a minimum cluster extent (k) of 3 contiguous voxels. We chose to use p values corrected for searching the whole brain volume to attempt to minimize Type I error, given the small sample size. Interpretation of imaging data focused on differences in targeted brain regions known to be involved in working memory processing based on prior functional imaging and lesion studies, including the prefrontal and parietal cortices and interconnected components of the attentional network shown to be activated in PET and fMRI studies (41, 42). Focusing on differences only in regions which constitute the brain network activated by this task and targeting our hypotheses to these areas also provides an additional means of minimizing Type I error.

Results

Performance Data

N-back scores were adjusted for false alarms with the following formula: adjusted score = proportion(Hits) – proportion(False Alarms). This formula corrects for subjects adopting a strategy where they endorse all items as targets. Data were analyzed with a 2 (drug versus placebo) X 4 (working memory load) ANOVA for SCOP and MECA separately. The expected effects of working memory load were found in both the SCOP ($F(3,15) = 4.19, p < .05$) and MECA ($F(3,15) = 24.0, p < .001$) analyses. No interactions of drug and working memory load were found for SCOP ($F(3,15) = .44, p > .70$) or MECA ($F(3,15) = .33, p > .80$). Neither SCOP nor MECA impaired performance relative to placebo on the N-back task ($F(1,15) = .14, p > .7, F(1,15) = .95, p > .3$, respectively).

Neuroimaging Data

Performance was examined on the most difficult condition of the N-back task, the 3-back condition, compared to the 0-back control condition, as the more difficult working memory load conditions have proved most sensitive to group differences in our prior studies. Activation related to SCOP and MECA challenges was examined separately as was done for the performance data. Across drug conditions (SCOP, MECA, placebo), the expected pattern of bifrontal, biparietal, and bicerebellar activation was seen for activation during 3-back relative to 0-back, consistent with our prior work (34-38). To determine the effects of anticholinergic challenge on working memory circuitry, we examined brain activation patterns for each drug relative to placebo in three different ways. First, we examined the drug-induced reduction in brain activation by comparing placebo relative to each drug condition. We hypothesized that regions with decreased activation on drug relative to placebo represent those areas where anticholinergic challenge results in diminished working memory-related brain activation. Second, we examined whether regions existed with increased activation under each drug relative to placebo suggesting compensatory activation of neural circuitry recruited to maintain task performance during anticholinergic challenge. Third, we directly compared MECA to SCOP to examine the differences in anti-nicotinic and anti-muscarinic drug challenge on working memory-related brain activation. These contrasts are described below, with results from paired t-test comparisons between drug conditions (MNI coordinates, cluster extent, and region descriptions) presented in Table 1. As noted above, results discussed below and in Table 1 include only those regions with p_{corr}

<.05. While other clusters of activation can be observed in the figures, these did not survive statistical correction for the whole brain search volume.

The potential impairing effect of the drugs was assessed by examining brain regions with less activation during each drug challenge relative to placebo. Diminished activation during MECA challenge relative to placebo was seen in the right medial frontal gyrus and right superior frontal gyrus (Figure 1). A similar effect was found for SCOP challenge in which there was less activation for SCOP relative to placebo in the right middle frontal gyrus and the left precuneus (Figure 2). Overall, the distribution of regions with diminished activation on drug (SCOP or MECA) relative to placebo was similar, though more pronounced for SCOP than for MECA.

The potential for compensation during anticholinergic drug challenge was assessed by examining regions with greater activation during drug challenge relative to placebo. Significantly greater activation was found in the right parahippocampal gyrus during the MECA challenge relative to placebo. No other regions showed significantly greater activation on drug (either MECA or SCOP) than placebo.

The differential effects of nicotinic versus muscarinic challenge on working memory-related brain activation during the 3-back were examined using a paired t-test in which two images per subject were entered into the model. Comparing the 3>0 back contrast image for the MECA and SCOP challenges allowed direct comparison of brain areas activated after nicotinic versus muscarinic blockade. In these analyses, greater activation on MECA relative to SCOP was found in the left middle frontal gyrus, while greater activation while on SCOP relative to MECA was found in the right superior parietal lobule and in the left cingulate gyrus (Figures 3A and B). Thus, both muscarinic and nicotinic blockade resulted in specific alterations in brain activation in working memory circuitry relative to placebo, and the effects of these anticholinergic drugs appeared to be dissociable.

Behavioral Measures

Questionnaires were completed by the participants and experimenter after returning from the MRI suite to assess whether there were any negative effects of the anticholinergic drugs or fMRI session on mood and physical symptoms. Similar to the performance data analysis, the behavioral data were analyzed separately for SCOP and MECA relative to placebo challenge. Overall, there were no effects of either SCOP or MECA relative to placebo on the subjective measures: the POMS, the Stanford Sleepiness Scale, the SVAS, and the PSCL. On the experimenter-completed OVAS the expected effects of SCOP were observed with subjects being rated as more drowsy ($t(5) = 3.79, p < .05$), more fatigued ($t(5) = 2.61, p < .05$), and less alert ($t(5) = 2.60, p < .05$) relative to placebo. There were no effects of MECA relative to placebo observed on the OVAS.

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. Only two significant changes were observed, both of which were expected. SCOP was associated with a higher pulse rate relative to the placebo condition ($t(5) = 4.05, p < .01$). MECA was associated with a significantly greater decline in systolic blood pressure relative to the placebo condition ($t(5) = 3.21, p < .05$).

Discussion

This study is the first to directly compare the effects of anticholinergic blockade of nicotinic and muscarinic systems during a working memory task using fMRI. Our data showed that

challenges to the cholinergic system resulted in diminished frontal lobe activation during drug challenges relative to placebo during a working memory task. A pattern of diminished activation on drug relative to placebo was found for both MECA and SCOP with differing specific frontal regions affected by each of the drugs. Overall, this may demonstrate that the activity of brain regions involved in performance of the N-back task was impaired by anticholinergic blockade. Performance on the N-back task was not significantly impaired by the cholinergic antagonists. It is possible that the lack of a significant effect of the drugs on performance may be secondary to the small sample size. However, our findings are consistent with that of Green et al (24) who similarly saw little performance effects of cholinergic antagonists on N-back performance in younger adults. These data may therefore lend support to a model that proposes that impairment of the cholinergic system leads to reduced ability to activate frontal regions typically involved in working memory processing.

We propose that in our postmenopausal sample the recruitment of additional frontal brain areas may be a result of activity of the cholinergic system. Our data showed that when the cholinergic receptors are partially blocked, frontal regions are less active relative to placebo. We are unable to differentiate whether the frontal deficits seen in this study are a result of age- or menopause-related effects on brain activity. If menopause has similar and/or additive effects to aging on cholinergic system activity and subsequent influences on task-related brain activity, then similar deficits may be produced.

The data in the current study can be compared with data from Saykin et al. (37), who examined the effects of donepezil, a cholinesterase inhibitor that increases both nicotinic and muscarinic signaling, on N-back performance in subjects with mild cognitive impairment (MCI). They found increases in frontal activation in MCI subjects after donepezil treatment relative to control subjects without MCI. In these patients with MCI, frontal regions were recruited during this task with the aid of the pro-cholinergic drug, suggesting that pharmacological manipulation counteracted dysfunction of the cholinergic system to allow successful performance of the working memory task. In our cognitively normal older women, we temporarily impaired the cholinergic system with anticholinergic drugs, and found decreased activation relative to placebo in similar frontal regions to those found in Saykin et al. (37). Taken together, data from the current study and Saykin et al. (37) support the idea that the cholinergic system is important for successful working memory performance and illustrate that cholinergic influences on task-related effortful processing can be examined with fMRI.

In addition, we found a dissociation for these activation patterns for nicotinic versus muscarinic challenges. Studies comparing nicotinic and muscarinic effects on the same cognitive process have been few. In a series of studies, Nathan and colleagues (24, 43, 44) and Little et al. (1998) have examined the effects of separate and combined nicotinic and muscarinic receptor antagonism on attention, early information processing, and working memory (24, 43, 44) in humans. Muscarinic blockade consistently produced larger effects than nicotinic blockade on working memory performance, but combined antagonism showed a greater magnitude of impairment than either drug alone, suggesting interaction of muscarinic and nicotinic mechanisms for optimal working memory performance. The potential mechanism(s) for these effects and interaction have been suggested by two prior neuroimaging studies. In a ¹³³Xenon inhalation study, Gitelman and Prohovnik (45) showed that scopolamine produced frontal cortex flow reduction that correlated with scopolamine-induced memory deficits and mecamylamine produced a perfusion deficit in parieto-temporal cortex. In a PET study of visual processing, Mentis and colleagues (46) were able to demonstrate that muscarinic activities predominated in primary and secondary visual processing areas (striate cortex, lateral visual association areas), whereas nicotinic

stimulation appeared to affect areas consistent with attention to the visual stimulus (thalamus and inferior parietal regions).

Prior functional imaging studies of nicotinic stimulation have strongly suggested nicotinic modulation of attentional processing. Thiel and colleagues (47, 48) and Lawrence et al. (49) showed a stimulus-specific modification of neural activity in parietal cortex consistent with improved signal-driven attentional performance on a visual-spatial orienting task (48) and visual processing tasks following nicotine. Interestingly, Kumari and colleagues (50) found that administration of nicotine during the performance of a verbal N-back task also showed parietal activity modulation and, as with our results with mecamlamine, found alterations in a distributed neural network which the authors proposed is related to online task monitoring and attention. Increased activation in the superior parietal region seen in the placebo versus MECA contrast from this study (Figure 1) is consistent with this interpretation.

Results of the present study are consistent with prior imaging studies in that there appeared to be separable effects of nicotinic versus muscarinic antagonism on working memory-associated brain activity. A hypothesis suggested by prior neuroimaging studies suggests that nicotinic and muscarinic systems may be responsible for different aspects of task performance; e.g., nicotinic stimulation may affect attentional modulation, rather than simply acting as a general signal gain enhancing system, whereas muscarinic effects may be more tied to stimulus processing or encoding (15, 20, 46). The results of the present study, although limited in their ability to dissociate subcomponents of task performance, tend to support this hypothesis, as the patterns of cortical activity shift were different between muscarinic and nicotinic antagonism compared to placebo rather than simply an alteration in the intensity of task-related activation. These results are also consistent with Sarter's proposal (4) that the cholinergic system optimizes signal driven detection (bottom-up) processes as well as top-down knowledge-based detection and filtering of irrelevant information. Nicotinic and muscarinic mechanisms may influence separate components of these processes and attentional changes in normal and pathologic aging.

Finally, working memory has been shown to be affected by the loss of circulating estrogen after menopause (51). The loss of estrogen during menopause and associated alterations in cognitive functioning may be a result of the effect of estrogen loss on cholinergic system functioning. We have shown that three months of estrogen treatment attenuated the effects of anticholinergic-induced impairment on cognitive task performance (52). Thus, future studies should directly examine the effects of the estrogen-cholinergic interaction on brain activation in postmenopausal women.

Some caveats should be considered with the interpretation of the current results. While the design of this study was an intensive within subjects design, this study had a small sample size, thus limiting the power to detect some effects of anticholinergic drugs on performance and related brain activity. Additional brain areas, particularly in the drug versus placebo comparison, may have survived correction with a larger sample size. In addition, we did not use a range of doses of the anticholinergic drugs. A larger range of doses would allow for differentiation of brain and performance effects of the different antagonists. However, in this pilot study the major focus was to identify separable effects of nicotinic and muscarinic blockade on brain activation and not on establishing dose response curves. Further studies will benefit from using a range of doses to better establish correlates between performance and brain activity. Also, we were not able to separate effects of menopause from aging in this study. To do so would require studying women on and off hormone therapy. Additionally, as our subjects were a small sample of healthy, highly educated postmenopausal women not on hormone therapy, these results do not generalize to the

population of all older women. Finally, the N-back task is a complex working memory task that does not allow for the dissociation of attentional, storage and manipulation components of the processes necessary to perform the task. Tasks that allow for these dissociations will need to be examined in order to differentiate the involvement of nicotinic and muscarinic systems on attention and memory processes.

In summary, this preliminary study of the effects of anticholinergic drug challenge on brain activity in postmenopausal women showed areas of decreased activation in the frontal lobe under cholinergic blockade which were different for muscarinic versus nicotinic blockade. We interpret these data to suggest that both nicotinic and muscarinic systems are important for the performance on working memory tasks and contribute differentially to task performance. Our subjects were postmenopausal women who were not taking hormone therapy and thus had low levels of circulating estradiol. We have previously shown that estrogen treatment attenuates the effects of anticholinergic challenge on tests of attention (52) and thus estradiol status may be important in influencing the activity of the cholinergic system. A recent study by Smith et al. (53) demonstrated the influence of estrogen and progesterone treatment in postmenopausal women on brain activation during a working memory task. This study found differences in brain activation between the hormone and placebo condition in frontal regions typically involved in working memory tasks. Thus, estrogen effects on brain activation patterns are important to examine in postmenopausal women and future studies should examine effect of the estrogen- cholinergic system interaction on specific cognitive operations using fMRI.

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PLC > MECA

Figure 1. Statistical parametric maps showing areas with greater activity on placebo relative to MECA challenge ($p < .05$) displayed over the MNI template surface rendering. See text for results of statistical analyses. R = right, L = left, A = anterior, P = posterior.

PLC>SCOP

Figure 2. Statistical parametric maps showing areas with greater activity on placebo relative to SCOP challenge ($p < .05$) displayed over the MNI template surface rendering. See text for results of statistical analyses. R = right, L = left.

Figure 3. Statistical parametric maps showing areas with greater activity on MECA relative to SCOP (A) and SCOP relative to MECA (B) ($p < .05$) displayed over the MNI template surface rendering. See text for results of statistical analyses. R = right, L = left.

Table 1

Placebo and drug paired *t*-tests: MNI coordinates, cluster extent, region descriptions (Brodmann's area, BA) and corrected *p* value.

Contrast	MNI Coordinates			Cluster Extent	Region Description	<i>p</i> _{corr}
	X	Y	Z			
PLC>MECA						
	4	-22	60	1005	Right Medial Frontal Gyrus (BA 6)	<.0001
	8	62	38	562	Right Superior Frontal Gyrus (BA 9)	.002
PLC>SCOP						
	24	60	26	1767	Right Middle Frontal Gyrus (BA 10)	.031
	-12	-58	30	4220	Left Precuneus (BA 31)	<.0001
MECA>PLC						
	10	-40	-4	502	Right Parahippocampal Gyrus (BA 30)	.005
MECA>SCOP						
	-26	-10	46	1324	Left Middle Frontal Gyrus (BA 6)	<.0001
SCOP>MECA						
	34	-56	54	947	Right Superior Parietal Lobule (BA 7)	.002
	-6	-44	42	640	Left Cingulate Gyrus (BA 31)	.034