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Enhanced Neuroactivation during Verbal Memory Processing in Postmenopausal Women Receiving Short Term Hormone Therapy

Carol C. Persad, Ph.D.^a, Jon-Kar Zubieta, M.D., Ph.D.^{a,b}, Tiffany Love, B.S.^{a,b}, Heng Wang, M.S.^{a,b}, Anne Tkaczyk, M.S.^c, and Yolanda R. Smith, M.D., M.S.^c

^aDepartment of Psychiatry, Medical School, University of Michigan, Ann Arbor, Michigan

^bMolecular and Behavioral Neuroscience Institute, Medical School, University of Michigan, Ann Arbor, Michigan

^cDepartment of Obstetrics and Gynecology, Medical School, University of Michigan, Ann Arbor, Michigan

Abstract

Capsule—Using a randomized, double-blind placebo-controlled cross-over design, we showed that short-term hormone replacement therapy increases brain activation in parietal and prefrontal areas during verbal memory tasks in postmenopausal women.

Objective—To study the effects of hormone therapy on brain activation patterns during verbal memory in postmenopausal women.

Design—A randomized, double-blind placebo-controlled cross-over study was performed.

Setting—A tertiary care university medical center.

Participants—Ten healthy postmenopausal women (age range 50–60 years) were recruited from the local community.

Interventions—Women were randomized to the order they received combined hormone therapy, 5 ug ethinyl estradiol and 1 mg norethindrone acetate, and placebo. Volunteers received hormone therapy or placebo for 4 weeks, followed by a one month washout period, and then received the other treatment for 4 weeks. An fMRI was performed at the end of each 4 week treatment utilizing a verbal memory task.

Main Outcome Measure—Brain activation patterns were compared between hormone therapy and placebo.

Results—Hormone therapy was associated with increased activation in left middle/superior frontal cortex (BA 6,9), medial frontal cortex and dorsal anterior cingulate (BA 24,32), posterior cingulate (BA 6), and left inferior parietal (BA 40) during memory encoding. All regions were significant at $p \leq 0.05$ with correction for multiple comparisons.

Conclusions—Hormone therapy increased neural activation in frontal and parietal areas in postmenopausal women during a verbal memory task.

Corresponding author and author to address reprint requests: Carol C. Persad, Ph.D. Department of Psychiatry, University of Michigan Health Systems, 2101 Commonwealth, Suite C, Ann Arbor, Michigan 48105. Telephone: (734) 763-9259 FAX: (734) 936-9262 cpersad@umich.edu.

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Keywords

Brain; women's health; neuroimaging; fMRI; verbal memory; estrogen; progestin; hormones; menopause

INTRODUCTION

Studies have demonstrated that hormone therapies (HT) can have beneficial effects on cognition in postmenopausal women. These findings have been seen with both estrogen (ET) and estrogen-progesterone (EPT) therapy regimes. Improvements in a number of cognitive domains including memory, language, executive functioning and speed of processing have been shown in women who undergo HT compared to age matched controls (1-4) although not all studies have reported positive findings (5-6). Though the recent findings of the Women's Health Initiative Study of Cognitive Aging (WHISCA) found mild declines in verbal memory associated with long term EPT, improved performance in visual memory skills was observed in the same women (7) suggesting some beneficial effects of estrogen on cognition.

Despite evidence of the potential benefits of estrogen, the underlying mechanisms of neurocognitive functioning remains poorly understood. Evidence from animal models has found estrogen receptors in numerous sites throughout the brain including the hippocampus, amygdala, hypothalamus, brainstem and cerebral cortex suggesting that estrogen therapy may impact cognitive functioning through potential effects on these brain areas (8). For instance, estrogen has been shown to have a direct effect on the hippocampus by increasing synaptic density in CA1 cells (9). Hippocampal structures are important in laying down memory traces for episodic memory as well as memory retrieval and changes in this area may underlie the observed memory improvements in women on HT. The prefrontal cortex (PFC) is also an area that is a major focus of estrogen action and is known to be involved in aspects of working memory, learning and retrieval, and attention (10, 11). Further, estrogen has been suggested to modulate various neurotransmitters (4, 12), increase cerebral blood flow (13-14), regulate the formation of synapses, affect neuronal survival (15-16), influence the expression of APOE and provide neuroprotective effects, all of which may either directly or indirectly impact cognitive functioning (15). However, not all research has supported the beneficial role of HT and recent research from the Women's Health Initiative studies has suggested that long term use in older women may result in increased risk of dementia (17).

Neuroimaging techniques have been helpful in understanding the effects of estrogen on brain functioning in vivo. Techniques such as Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI) can provide information regarding brain regions and neurotransmitter systems involved in cognitive processes. With PET, observed increases in glucose metabolism during cognitive tasks have been used to infer increased neuroactivation in those brain regions associated with the cognitive activity. One PET study found that women using HT (either ET or EPT) when compared to a group of women who were not on any hormones, showed increased glucose metabolism in the right parahippocampal, right frontal and right precuneus regions as well as the left hypothalamus during a verbal memory task, while the right parahippocampal area, left visual association areas, and inferior parietal and anterior thalamic regions showed greater activation during a visual memory task (18), suggesting HT enhanced neuroactivation in these areas. PET can also be used to isolate specific neurotransmitter activity. For instance studies have shown increased serotonin receptor binding associated with HT in the PFC (12, 19).

Functional magnetic resonance imaging (fMRI) is a less invasive technique to look at neuroactivation. fMRI detects changes in the blood oxygen level dependent signal (BOLD), believed to reflect changes in neural activity. Using both visual and verbal working memory tasks, Shaywitz and colleagues found increased activation bilaterally with fMRI in the superior frontal gyrus and the inferior parietal lobule in subjects using high dose conjugated equine estrogen compared to placebo (20). Joffe and colleagues (21) found similar activations with estradiol treatment in the frontal and parietal areas during a verbal memory task, while increased activation in the frontal regions as well as the anterior and posterior cingulate was found during a spatial memory task.

As part of a comprehensive evaluation of EPT on brain functioning, we previously reported increased bilateral prefrontal activation with fMRI during a spatial working memory task in postmenopausal women (22). Here we present the effect of EPT on neural pathways involved in verbal memory. A randomized double blind placebo controlled crossover design was used to examine the effects of EPT on brain metabolism during a verbal encoding and delayed recognition task. It was hypothesized that greater activation of hippocampal regions and the prefrontal system would occur during the EPT condition compared to placebo during the verbal memory task.

MATERIALS AND METHODS

Subjects

Ten healthy right handed postmenopausal women (age range 56-60 years) were recruited from the community to participate in a comprehensive evaluation of hormone therapy. Demographic information is presented in Table 1. All women underwent spontaneous natural menopause defined as the absence of menstrual cycles for at least 1 year. Verbal learning and memory performance was within normal limits when compared to an age normative sample (HVLT-R; 24). Further, no evidence of depressive symptoms was evidenced on a self report measure of depression (GDS; 25).

All subjects underwent physical exams including pelvic examination, ultrasound, and extensive medical and psychiatric history taking. Exclusion criteria included the presence of a significant neurological, psychiatric or medical disorder, history of head trauma with loss of consciousness, or history of substance abuse. In addition, the women could not have received HT in the last 3 months prior to the study. Informed consent was obtained at the initial visit. This study was approved by the University of Michigan Medical School Institutional Review Board.

Study Design

A randomized double blind placebo controlled crossover study design was used to evaluate the effects of EPT on brain activation patterns during a verbal memory task. After an initial baseline evaluation that included a neuropsychological assessment to rule out the presence of cognitive difficulties, subjects were randomized to either placebo or EPT administration. Hormone therapy consisted of 5 ug ethinyl estradiol and 1 mg norethindrone acetate (Femhrt, Warner Chilcott, PLC, United Kingdom). Subjects received EPT or placebo for 4 weeks, followed by a 4 week washout period, and then received the other treatment for 4 weeks. At the end of each treatment (EPT or placebo), subjects underwent an fMRI study consisting of verbal memory, visual memory and emotional processing tasks (22). The results of the verbal memory task are presented here. Pill counts were done at the end of each treatment condition to document compliance.

Verbal Memory task

The levels of processing (LOP) paradigm was used to examine episodic verbal memory processes. In the typical LOP paradigm individuals are asked to judge a list of words based on word characteristics. Judgments are designed to orient the subject to either the physical characteristics (shallow processing) or the meaning of the words (deep processing). Subjects are not told in advance of a later recognition memory task (referred to as an incidental memory task). During the recognition memory task, typical results consistently show better recognition for words originally processed at a “deep” vs. “shallow” level. The LOP effect has been extensively studied and shown to be mediated by the hippocampus and PFC in both lesion as well as neuroimaging studies (23).

Verbal Encoding Phase—32 lists consisting of 12 words each were equated for letter length and frequency based on Kucera-Francis written frequency of 10-100 from the Medical Research Council Psycholinguistics database (http://www.psy.uwa.edu.au/uwa_mrc.html). Presentation of the lists was counterbalanced for each subject across the placebo and treatment conditions. Word lists were presented through RF-shielded goggles mounted to a headcoil (Resonance Technology Inc., Northridge, CA). During the encoding phase, word lists were presented in a blocked design across 4 scanning runs. In each run, 4 lists were presented. Prior to each list presentation, subjects were shown a set of instructions (8 second duration). Word presentation was 1.5 seconds with a 1.5 second interstimulus interval. In the phonemic condition, subjects decided if the word presented was in capital or lowercase letters (shallow processing). In the semantic condition (deep processing) subjects decided whether each word referred to an abstract or a concrete object/concept. Instruction set (semantic vs phonemic presentation) was counterbalanced across each scan period, and for each scan run, 2 lists required semantic judgments and 2 required phonemic judgments. Each list consisted of equal numbers of uppercase and lower case as well as concrete and abstract words. Responses were made by button press, with the index and middle finger of the right hand. Subjects were not informed that their memory for the words would be tested at a later time during the session. Stimuli were presented with a commercial software package (E-Prime, Psychology Software Tools Inc., Pittsburgh, PA). Accuracy and response time were recorded.

Verbal Recognition phase—After a 20 minute delay, during which an unrelated visual spatial working memory task was administered, a recognition test was presented. This task was done in 2 scan periods. Each word was presented for 1.5 seconds, with a 1.5 interstimulus interval. Ninety six words were shown in each run, half of which were previously presented words and half were new (i.e never presented) words. Subjects were required to make a yes/no decision by button press indicating whether each word was presented during the initial encoding phase. Half of the previously presented words were from the phonemic condition, and half from the semantic condition.

General procedure—Prior to the initiation of the imaging experiment, all subjects were trained on the verbal encoding task using computer-based presentation software identical to that employed during the study, until the subjects were comfortable with all the procedures and achieved an accuracy rate of at least 70%.

FMRI Acquisition and Processing

All scans were acquired using a 3T whole body MRI scanner (General Electric, Milwaukee, WI) equipped with a standard headcoil. Anatomical MRI scans were acquired axially in all subjects with an SPGR 3D volumetric acquisition (TR 9.6 / TE 3.3 / IR PREP 200ms / flip angle 17 degrees / bandwidth 15.63 / 24cm F.O.V. / 1.5mm slice thickness / 100 or 106 slices / 256 × 256 matrix / 2 nex) for anatomical localization and coregistration to

standardized stereotactic coordinates. fMRI acquisition was sensitized for the BOLD effect using a T2* weighted single-shot spiral pulse sequence (26) with 32 oblique-axial slices prescribed to be approximately parallel to the AC-PC line (Spiral GRE, TE = 25, TR = 2000, FA = 60°, 4 mm-thick contiguous slices, 24 cm FOV, image matrix = 64 dia). Image reconstruction included processing steps to remove distortions caused by magnetic field inhomogeneity and other sources of misalignment to the structural data (26). Functional images were processed with the slice-timing procedure; each image was sinc-interpolated in time, slice-by-slice, to correct for the staggered sequence of slice acquisition (27).

All fMRI data analyses were conducted using SPM2 (Wellcome Department of Cognitive Neurology, London, UK). Images were realigned first to the volume of the first run using SPM2-based algorithms (28). The first four functional volumes of each run were discarded to remove magnetic saturation effects. Realignment parameters for each subject were examined to ensure subject's head movement did not exceed 2 mm.

Subject's 3D structural and functional images were coregistered to each other and then spatially normalized into standard stereotactic space (International Conference on Brain Mapping (ICBM) stereotactic space) via non-linear warping using image processing tools within SPM2. A three-dimensional Gaussian smoothing kernel set at 8 mm full-width-at-half-maximum (FWHM) was applied to each subject's functional data to accommodate for residual anatomical variability and to improve signal to noise ratios. For each subject, each of the conditions were modeled as epochs and planned comparisons computed as linear contrasts. The motion parameters collected during scanning were used in the individual analyses as regressors. The anatomically standardized contrast *t* maps for each subject were then entered into a second-level paired *t*-test analyses for the examination of treatment effects. Areas of activation were deemed significantly different between these two groups if they included at least 20 voxels and reached a statistical threshold of $p < 0.05$, corrected for multiple comparisons and spatial extent (Friston et al., 1994).

RESULTS

Behavioral Data

During the encoding phase, accuracy of the semantic judgments was 76% (S.D.=13) during the placebo condition and 78% (S.D. =14) during EPT. Accuracy of the phonemic judgments was 95% (S.D. = 7) and 97% (S.D. = 2) for the placebo and EPT conditions, respectively. No performance differences between the EPT and the placebo condition was found. Reaction times for the phonemic (shallow) judgments were faster (0.72 secs, S.D.=0.09) than the semantic (deep) judgments (0.98 secs, S.D.=0.13). As expected, recognition of words that were encoded semantically (deep encoding) was much higher than phonemically encoded words (shallow encoding; $t=7.443$, $p<0.000$). There was no difference between the placebo and EPT conditions in recognition performance. Sixty-one percent (S.D.=25) and 58% (S.D.=28) of the words from the semantic lists were correctly recognized for the placebo and HT conditions, respectively. In contrast, 24% (S.D.=15) and 28% (S.D.=19) of words were recalled from the phonemic lists.

Neuroimaging results

To first demonstrate an effect of task, contrast images were generated for each subject to assess differences in activation between the semantic and phonemic encoding conditions. The contrast images were placed into ICBM stereotactic space using the transformation matrix derived from the linear and non-linear warping transformation matrices (described above). Table 2 demonstrates the brain areas activated by this task during deep encoding compared to shallow encoding for all subjects. Increased activation was seen in the left

frontal region encompassing the dorsolateral PFC, the medial PFC (primarily the anterior cingulate area), dorsal anterior cingulate, right middle temporal gyrus, right insula, left putamen, bilateral occipital cortex, bilateral parietal cortex areas encompassing the angular gyrus, and the cerebellar vermis, consistent with prior literature for this task (Figure 1, (23)). No significant activations were found in the shallow encoding condition when compared to the deep encoding condition.

To evaluate the effect of EPT, the contrast images for each subject during hormone therapy and placebo conditions were analyzed at the group level using both paired and two-sample t-tests for the semantic-phonemic contrast. Numerical differences between groups were determined by averaging the values of voxels contained in an area of significant differences. These data were then plotted and examined for regional differences between conditions to eliminate the possibility that significant effects in the voxel-by-voxel comparisons were caused by artifactual data or outliers.

Table 3 presents the comparisons of the EPT minus placebo condition. Compared to placebo, increased activation during EPT was seen in the left and medial areas of the PFC, dorsal anterior cingulate, posterior cingulate and left parietal cortex (Figure 2). Looking at the reverse contrast (placebo minus EPT), there were no areas of increased activation in the placebo condition compared to the EPT condition. Due to the relatively low recognition rate of the subjects in conjunction with the event related design of the recognition task, there was not enough power to analyze brain activation during the recognition phase.

DISCUSSION

Consistent with earlier studies, our results showed regional increases in fMRI BOLD signal during a verbal memory task while on short term hormone therapy compared to placebo in postmenopausal women. Specifically, increases in activation were found in the prefrontal and parietal cortices. Prefrontal and parietal activation has been demonstrated in previous studies examining episodic semantic memory processes using paradigms such as the levels of processing task (23, 31, 32). In our group of women, EPT enhanced activation in these brain regions, suggesting that short-term HT may be beneficial to brain functioning. The anterior cingulate, postulated to be important for the processing of lexical information and associative cognitive and decision making processing (33), also showed increased activation with EPT. This is expected given the need for specific decision making in the verbal encoding task.

In contrast to expectations, we did not see significantly increased activation in the hippocampal or parahippocampal regions that are typically associated with deeper encoding processes. Activations were present in this area although the difference between the two treatment conditions did not reach statistical significance after full corrections for multiple comparisons. It is feasible that changes in the hippocampal and parahippocampal regions are more sensitive to performance differences and only are seen on neuroimaging when a measured difference in behavior is documented, unlike the findings from this study. In addition, our relatively small sample size may have also accounted for the lack of significant differences in this area. Further, one could argue that behaviorally, performance on the recognition memory test was quite low, and therefore our power to detect activation was also low. Indeed, due to the low recognition hit rate and the event related design of the recognition task, we did not have enough power to analyze the recognition data. It is possible that the intervening task used between the initial encoding and recognition task may have influenced recall in some manner, possibly due to interference or fatigue effects, although it was an unrelated task. However, a number of factors speak against this interpretation. First, in comparison to other studies using a LOP paradigm, similar patterns

in performance data were reported, even when shorter word lists or easier to remember stimuli were used (34) suggesting that our verbal memory paradigm worked appropriately. More likely, the lack of significant findings in the hippocampal and parahippocampal region reflects a change in neuroactivation that occurs as part of the aging process. Relative increases in activation in the frontal cortex with associated decreases in temporal cortex areas (including the hippocampus) have been shown to occur in older adults during a range of cognitive tasks including memory, and our findings are consistent with those data (34-35).

Other studies have demonstrated increased brain activations, particularly in the PFC, with HT in postmenopausal women using both verbal and visual working memory tasks (c.f. 20,21), although the areas of PFC activation did not overlap with our findings. However, this is likely a function of the memory task used in the different studies. This study is the first to demonstrate increased neuroactivation during semantic memory processing using the LOP paradigm with EPT. Semantic memory and working memory tasks have been shown to activate different areas, although the PFC is involved in both memory systems. The fact that increased neuroactivations are evidenced regardless of the memory task, suggests that HT acts generally on brain circuits used in memory processing. Research in the animal literature also supports the concept of an enhanced memory system with estrogen mediated through increased action in the PFC. Indeed spatial working memory processes have been shown to be enhanced with estrogen in ovariectomized aged rhesus monkeys (36) and rats (37). These findings suggest that the role of estrogen may be to increase the overall efficiency of neural circuits that are typically recruited for cognitive tasks, perhaps through modulation of different neurotransmitter systems such as serotonin (12, 19), acetylcholine (4) or dopamine (38) that are known to influence the hippocampal and prefrontal regions.

It is of interest that activation changes were observed in the absence of differences in the behavioral data (i.e. recognition memory performance was equivalent in the two conditions). This finding is consistent with others who have demonstrated similar results (20). This may reflect increased sensitivity of functional neuroimaging techniques to HT effects on the brain compared to traditional behavioral measures.

Not all studies have shown that HT is beneficial to cognition and in fact the Women's Health Initiative Memory Study (WHIMS), questioned the effectiveness of HT use, as their longitudinal data suggested an increased risk of cognitive impairment and vascular related dementia in longterm HT users (39). However, in light of recent data, researchers have suggested that the timing of HT therapy in postmenopausal women may be a key factor in understanding HT's affect on cognition (40-42). In a review of the available literature, Maki (41) showed that the majority of studies found benefits associated with estrogen use in verbal memory and attention processes in women less than 65 years of age, while findings were inconsistent in studies that used older women. As a result of these findings, it has been suggested that estrogen may provide cognitive benefits when given to younger women shortly after or during the menopausal transition. Compared to the WHIMS study whose participants were all over the age of 65, our subjects were much younger (in their fifties), which may account for the discrepant results.

One strength of this study is the use of a crossover, placebo controlled experimental design. The finding of increased activation during the EPT condition compared to placebo in the same group of women strengthens the argument that estrogen has a beneficial effect on neural functioning, as individual differences between treatment groups generally seen in traditional cross-sectional designs were eliminated in this study. However, the use of combined estrogen and progestin therapy does not allow us to tease out the individual effects of estrogen and progestin on neural functioning. Varying dose and combination of

hormones are likely to have differential effects on hormone receptors. Further studies are necessary to help elucidate the specific effects of estrogen.

In summary, our results demonstrate that short term hormone therapy results in changes in neural activation in verbal memory circuits in postmenopausal women and suggests that estrogen may enhance the overall efficiency of verbal memory processes in postmenopausal women.

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Figure 1. Brain regional activation during deep encoding with respect to shallow encoding

Images display areas where greater BOLD activity was detected during the deep encoding trials relative to the shallow encoding trials for all subjects. Images are displayed in neurological convention (right of the image is the right side of the brain) at a display threshold of $p < 0.001$. Significance levels are displayed using the color scale on the bottom of the figure and are superimposed over anatomical MRI images in ICBM coordinates.

Figure 2. Differential effect of hormone therapy with respect to placebo on deep encoding effects Images display areas where greater BOLD activity was detected when subjects were treated with hormone therapy, compared to placebo treatment during verbal encoding (deep encoding – shallow encoding contrast). Images are displayed in neurological convention (right of the image is the right side of the brain in coronal views, right is front of the brain in sagittal views) at a display threshold of $p < 0.001$. Significance levels are displayed using the color scale on the left of the figure and are superimposed over anatomical MRI images in ICBM coordinates.

Table 1

Demographic and Baseline Information

	Average (+/- SD)
Age (years)	56.9 (1.4)
Menopause Age (Years)	50.0 (2.7)
Years Post-Menopause	6.9 (2.8)
Baseline Estradiol (pg/mL)	17.2 (4.8)
Education (Years)	16.3 (1.9)
Shipley Estimated IQ	115.0 (12.0)
Mini Mental Status Exam (MMSE)	28.6 (1.9)
Geriatric Depression Rating Scale	0.4 (1.0)
Hopkins Verbal Learning Test-Revised (HVLTR) Total Learning	25.9 (2.8)
HVLTR Delayed Recall	10.1 (2.1)

Table 2

Effect of Task, Deep –Shallow Encoding

Region ^{a, b}	Coordinates (x, y, z) ^c	Z	Cluster Size ^d
Left Frontal Cortex (Including Middle and Inferior Gyri: BA 44,45,46, 47, 9)	-48, 24, 16	5.17	3699
Dorsal Anterior Cingulate (Extending into the Medial Frontal Cortex: BA 32, 24)	8, 14, 28	5.02	3214
Right Insula	42, 22, 0	5.37	4308
Left Parietal Cortex (BA 39)	-32, -70, 32	4.64	662
Left Parietal Cortex (BA 7)	-42, -36, 48	4.61	172
Right Parietal Cortex (BA 39)	30, -70, 32	4.16	341
Right Middle Temporal Gyrus (BA 21)	50, -36, -6	4.22	463
Right Occipital Cortex (BA 17, 18)	30, -90, 2	4.77	1385
Left Occipital Cortex (BA 17, 18)	-34, -88, -2	4.30	588
Left Putamen	-22, 4, 6	4.60	378
Cerebellum, Vermis	-2, -58, -44	4.17	133

^a Brain areas with significantly increased activation seen on fMRI in the deep-shallow encoding condition are listed in the table.

^b All regions significant at $p \leq 0.05$, corrected for multiple comparisons at the cluster level

^c Coordinates (x,y,z) refer to the ICBM coordinates where the significant differences were centered in mm.

^d Cluster size is expressed in voxels of 2 mm^3 .

Table 3

Effect of Hormone Therapy, Deep-Shallow Encoding

Region ^a	Coordinates (x, y, z) ^b	Z	Cluster Size ^c
Hormones > Placebo			
Left Prefrontal Cortex (BA 6,9)	-24, 16, 50	3.24	765
Dorsal Anterior Cingulate (Extending into the Medial Frontal Cortex BA 32, 24)	10, 12, 48	5.21	278
Posterior Cingulate (Extending into the Frontal Cortex (BA 6)	6, -18, 60	3.36	364
Left Parietal Cortex (BA 40, 39)	-48, -32, 22	3.50	573

^a All regions significant at $p \leq 0.05$, corrected for multiple comparisons at the cluster level

^b Coordinates (x,y,z) refer to the ICBM coordinates where the significant differences were centered in mm.

^c Cluster size is expressed in voxels of 2 mm³.