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Low-dose estradiol alters brain activity

Michael C. Stevens[a,b,*], Vincent P. Clark[c], Karen M. Prestwood[d]

[a]Clinical Cognitive Neuroscience Laboratory, Olin Neuropsychiatry Research Center, Whitehall Building,
The Institute of Living/Hartford Hospital, Hartford, CT 06106, USA
[b]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA
[c]Department of Psychology, University of New Mexico, Albuquerque, NM, USA
[d]Center on Aging, University of Connecticut Health Center, Farmington, CT, USA

Abstract

Although several studies have examined the effects of estrogen replacement therapy (ERT) on neural activity associated with tasks of learning and memory, no study has examined such effects on a sustained attention task. This study examined the effect of low-dose estrogen replacement therapy on hemodynamic activity elicited by a visual three-stimulus oddball task recorded using event-related functional magnetic resonance imaging (fMRI). Participants included 16 women between the ages of 73 and 84 who were part of a randomized controlled double-blind study to evaluate the effect of an ultralow dose micronized estradiol on bone. No significant differences in behavioral performance were found with ERT. However, there was evidence that ERT group participants had both reductions and increases in the amplitude of hemodynamic response in a variety of subcortical and cortical brain regions. These included regions involved in perception and attention such as the occipital and parietal lobes, motor cortex, anterior cingulate and prefrontal cortex. These findings suggest that estrogen may facilitate the efficiency of brain function during the performance of sustained attention tasks in post-menopausal elderly women.
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1. Introduction

Estrogen is a gonadal steroid hormone that exerts wide-ranging effects on the central nervous system. A review of studies that examined the effect of estrogen replacement therapy (ERT) on cognitive function (Yaffe et al., 1998) found mixed results. Some studies showed no effect, some had equivocal results, and still others reported significant improvements to cognitive function following administration of estrogen. It is possible that differences among studies might be the result of different ERT formulations, doses, and routes of ERT administration, or might reflect sample characteristics such as duration of ERT or subject age.

* Corresponding author. Clinical Cognitive Neuroscience Laboratory, Olin Neuropsychiatry Research Center, Whitehall Building, The Institute of Living/Hartford Hospital, Hartford, CT 06106, USA. Tel.: +1 860 545 7552; fax: +1 860 545 7997.
E-mail address: msteven@harthosp.org (M.C. Stevens).

Alternatively, the variability in results could be related to the complex and heterogeneous nature of cognitive function. Consistent with this idea, a recent meta-analytic review of ERT neuropsychological studies concluded that estrogen does not affect all cognitive domains equally (LeBlanc et al., 2001). It appears to improve cognitive performance on tests of verbal memory, vigilance, reasoning and motor speed in peri- and post-menopausal women who also have other symptoms of menopause.

Several neuroimaging studies indicate that hormone replacement therapy alters brain activity associated with cognitive performance. These studies have used positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to detect differences in the spatial extent or magnitude of brain activity between ERT and non-ERT groups. These methods have been used to study both resting state metabolism and neural activity elicited during the performance of specific cognitive tasks, most often those tasks that examine memory function. In a PET study comparing three groups of postmenopausal women, including women on ERT, women not on ERT, and women with Alzheimer's Disease, Eberling et al. (2000) found that estrogen administration resulted in higher resting state glucose metabolism in several brain areas, including bilateral dorsolateral prefrontal cortex, bilateral middle temporal gyrus, and bilateral inferior parietal lobule in women who were on ERT. These brain areas are associated with both memory function and cortical degeneration in Alzheimer's Disease. Another study found that women on ERT had better performance on a verbal memory test and changes in regional cerebral blood flow (rCBF) during verbal and nonverbal memory tests (Resnick et al., 1998). On the verbal memory test, women on ERT had decreased rCBF in right precuneus, parahippocampal gyrus, and dorsal frontal gyrus, while women not on ERT showed increased rCBF in right inferior frontal cortex. On the visual memory test, women on ERT again showed deactivation of the right parahippocampal gyrus. This study is noteworthy in that its results do not permit easy interpretation; rather, Resnick et al. (1998) found evidence for task × group interactions, suggesting that estrogen's influence on brain activity is complex. Maki and Resnick (2000) followed up these women 2 years later and found that women on ERT showed increased rCBF during rest or task performance in hippocampus, parahippocampal gyrus, and temporal lobe areas in association with continued ERT.

Several other studies found evidence that estrogen altered neural activity during the performance of tasks involving attention and executive control of cognitive processes. Berman et al. (1997) depleted young women of endogenous estrogen using Lupron (a gonadotropin-releasing hormone agonists that decrease the body's production of specific hormones) and examined rCBF during the Wisconsin Card Sort Task, which involves several complex functions typically defined as "executive" in nature. Although there was no evidence for task performance impairments, depletion of estrogen resulted in reduced perfusion in prefrontal cortex, inferior parietal lobule and posterior inferior temporal lobe areas. Adding estrogen resulted in normalization of brain activity. Using fMRI, Shaywitz et al. (1999) examined brain function in postmenopausal women on verbal and nonverbal versions of the Sternberg working memory task. This working memory task involved short-term online maintenance of information prior to subsequent identification of previously seen items. Shaywitz et al. (1999) found that short-term ERT (i.e., 3 weeks) increased activation in prefrontal cortex areas (i.e., superior frontal gyrus), bilateral inferior parietal lobule, and right middle and superior occipital gyri during encoding. ERT also led to a decrease in inferior parietal lobule activity with nonverbal material. During retrieval from working memory, ERT increased hemodynamic activity in right superior, middle and inferior frontal gyri, posterior cingulate and precuneus. Decreased activity in the ERT group compared with the non-ERT group was seen in right superior temporal gyrus and insula and left central sulcus. Dietrich et al. (2001) examined brain activity in premenopausal women at different phases of their menstrual cycle. These women had increased activity in brain areas associated with word stem completion task performance (i.e., left inferior frontal gyrus, medial frontal gyrus, and postcentral gyrus) and mental rotation task performance (i.e., bilateral angular gyrus/inferior temporal-occipital cortex and superior parietal lobule) at times in the menstrual cycle associated with higher estrogen levels.

In sum, these neuroimaging studies demonstrate that on different cognitive tasks, modulation of estro-

gen levels affects neural activity in widespread brain structures. The effect of ERT appears to depend on the sensory modality of cognitive processing. Specifically, the evidence suggests that neural activity changes on verbal tasks may be accompanied by overt behavioral improvements, but that neural changes on nonverbal attention or executive tasks may not affect behavioral performance. Furthermore, while previous studies showed that ERT both increased and decreased neural activity, ERT did not appear to change the localization of activity. The fact that ERT was associated with both increases and decreases of rCBF and hemodynamic activity suggests that estrogen's effects on brain function may not be explained by a single mechanism. Rather, estrogen's effects on the brain likely involve mechanisms that modulate neural network activity, and assessments need to be made within the context of the task used to elicit brain activity. Therefore, it would be useful to characterize the effect of ERT on other cognitive processes besides memory, working memory, and executive control.

To date, the effect of ERT on neural activity associated with simple attention has not been studied. The purpose of this study is to use a common task of selective and sustained attention using event-related fMRI to determine the impact of ERT on brain function. The task used in this study is an fMRI version of a visual oddball paradigm. Oddball tasks frequently have been used to examine brain function using the electroencephalogram (Polich and Kok, 1995). These tasks employ frequently occurring standard stimuli and infrequently occurring task-relevant target stimuli. Participants are instructed to identify targets, usually with a button press, but to not respond to other stimuli. The processing of target stimuli is believed to be associated with stimulus evaluation/categorization (Donchin and Heffley, 1979), specifically with cognitive processes necessary to update mental models of context within working memory (Donchin and Coles, 1988). Some oddball versions also present infrequent task-irrelevant stimuli. Brain activity to rare task-irrelevant stimuli is thought to reflect an automatic orienting response, and has been seen to occur even in the absence of controlled attention. Over a dozen studies have been published using healthy adults as subjects in fMRI oddball tasks, with the majority of published work using visual stimuli (Casey et al., 2001; Clark et al., 2001, 2000; Jaencke et al., 2001; Kiehl et al., 2001; Kirino et al., 2000; Linden et al., 1999; McCarthy et al., 1997; Menon et al., 1997; Opitz et al., 1999; Strange and Dolan, 2001; Strange et al., 2000). These studies reveal that a large number of different cortical and subcortical areas show increased neural activity during the processing of target stimuli. In particular, target identification elicits increased hemodynamic activity in bilateral inferior parietal lobule and in superior, middle, and inferior prefrontal gyri. Therefore, it was hypothesized that ERT would be associated with differences in the amplitude of hemodynamic response in these areas. It additionally was predicted that ERT would modulate hemodynamic activity in precuneus and posterior cingulate. All of these structures have been shown in previous fMRI oddball studies to be engaged in oddball task processing, and all of these brain areas have shown changes in neural activity following ERT administration in previous neuroimaging research. Because previous research has found evidence for improvements in reaction time on cognitive tasks, it also was hypothesized that women on ERT would have faster reaction times compared with those on placebo.

2. Methods

2.1. Participants

Participants in the current study were 16 women between the ages of 73 and 84 years who were participating in a double-blind 3-year study of ultralow dose estrogen on bone density at the University of Connecticut Health Center. As part of this study protocol, participants were randomly assigned to one of two conditions: 1) 8 women took ultralowdose estrogen (0.25 mg/day), and 2) 8 women took placebo. Women with a uterus, regardless of treatment group, were given progesterone 100 mg per day for 2 weeks every 6 months. All women also received calcium citrate 1300 mg and 1000 IU vitamin D per day. The details of the entire cohort as well as recruitment efforts and reasons for drop-out were previously published (Prestwood et al., 2003). The first 60 women who enrolled in the bone density study were also

invited to participate in a cognitive sub-study. These women received extensive neuropsychological testing at baseline, at 3 months and at 3 years into the study. Of these 60 women, those who completed the entire protocol were invited to participate in the fMRI study prior to discontinuing treatment. Differences between the group's demographics were assessed by independent sample *t*-tests based on a two-tailed distribution or χ^2 statistics.

2.2. Materials

The fMRI experimental task was a three-stimulus visual oddball task adapted for fMRI (Clark, 2002; Clark et al., 2000). Stimuli were frequent standard (the letter "T", $P=0.82$), rare distractor (the letter "C", $P=0.09$), and rare target (the letter "X", $P=0.09$, to which subjects made a speeded button press response) single block letters, with inter-stimulus interval varied randomly from 550 to 2050 ms across trials. Stimuli were presented for 200 ms in a rapidly presented, pseudo-random order. A commercial software package (STIM) on an IBM personal computer located in the MRI control room was used to control the timing of the stimulus presentation. An LCD-based RF-shielded audiovisual presentation system was used for fMRI stimulus presentation (Resonance Technology, Los Angeles, CA). Participants viewed the images through a set of goggles, and wore a headphone/microphone set that allowed communication with experimenters in the MRI control room as well as dampened scanner noise. Manual responses were recorded through a four-button optical response device (Current Designs Inc., Philadelphia, PA) that was connected to a separate computer located in the MRI control room. This separate computer also received pulse-timing information from the MR scanner and event timings from the STIM computer for the overall coordination of MRI data acquisition, stimulus timing, and recording of subject responses. Participants were instructed to respond to rare targets (the letter "X") that appeared on the screen by making a speeded button press with their right thumb as quickly as possible. They were instructed not to make a response to any other stimulus. Participants were given an opportunity to practice the task once in the control room using a similar response device prior to beginning fMRI data collection.

2.3. fMRI procedures and data analysis

A Siemens Vision Magnetom MR system (Siemens Medical Systems, Erlangen, Germany) operating at 1.5 Tesla and equipped for echo planar imaging (EPI) was used to acquire functional MRI data and high-resolution structural images of the head. This machine is located at the University of Connecticut Health Center. Head position was determined using sagittal, axial, and coronal scout images (TR=500 ms, TE=10 ms, 192×256 matrix, duration=7 s). A gradient echo, echo planar scanning sequence was used (single-shot, 20 oblique slices collected in an interleaved manner, 6-mm thick, no skip, repetition time (TR)=2150 ms, echo time (TE)=40 ms, flip angle=90°, field of view=25.6 cm, matrix=64×64, with T2* weighted endogenous BOLD contrast). Slices were positioned so that the top slice covered the vertex of the cortex, resulting in nearly whole brain coverage. Some areas of cerebellum were not covered for subjects with above average brain size; however, all areas of cortex were included in the EPI images. This produced 90 EPI images for each functional MRI run (3 min 13 s per run). Participants underwent a eight to nine functional EPI runs. Some subjects received more runs when movement-related artifact was suspected. On half of the EPI runs, negative Z-shimming was used to compensate for magnetic inhomogeneity artifacts, thus increasing signal intensity obtained from orbital and ventromedial-prefrontal cortex (Constable and Spencer, 1999). As a result, an approximately equal number of Z-shimmed and non-Z-shimmed runs were obtained so that there would be adequate sensitivity to signal change throughout the cortex. For two Z-shimmed runs obtained from participants in the placebo group, there were inhomogeneity artifacts that prevented adequate coverage in superior brain slices. These runs were discarded. However, non-Z-shimmed runs for these subjects showed no distortion, and they were all used for analysis.

Movement correction and spatial normalization was performed using SPM99 (Wellcome Department of Cognitive Neurology, London, England). The first four EPI volumes were discarded to avoid saturation effects on subsequent preprocessing and statistical analyses. All remaining EPI volumes were registered to the first echo planar image of the sequence and

resliced using sync interpolation, resulting in 86 movement-corrected images in each run. Two runs from one subject had rotation in excess of $2°$ and were excluded from further analysis. All other runs' movement correction measures were within 2 mm and $2°$. In order to perform inter-subject averaging, images were converted to a standard space by matching the EPI template image that permitted subsequent conversion of coordinates to the modified Talairach space used in SPM99. As a final preprocessing step, images were spatially smoothed to a final FWHM of 10 mm to compensate for residual variability after spatial normalization and to permit statistical inference using Gaussian random field theory (Friston et al., 1995). In addition, the data underwent a fifth-order IIR Butterworth low-pass filter of 0.16 Hz to remove high frequency noise associated with alterations in the applied radiofrequency pulses. The filtering served to improve signal-to-noise in the BOLD image time series.

Event-related responses to the target stimuli were modeled using a synthetic hemodynamic response function composed of two gamma functions (see Josephs et al., 1997, for mathematical model; and Friston et al., 1998, for illustration). The first gamma function modeled the hemodynamic response using a peak latency of 6 s. A term proportional to the derivative of this gamma function was included to allow for small variations in peak latency. The second gamma function and its associated derivative were used to model the small 'overshoot' of the hemodynamic response on recovery. The modeled composite hemodynamic response for target processing in each run was derived by extracting stimulus-onset timings for only those events that each participant responded to correctly (i.e., targets with correct button-presses after 200 ms, but within 1500 ms, correctly ignored standard or distractor stimuli). Thus, every subject had an fMRI time-series model specific to her exact behavioral response patterns. Because the number of error trials was small (less than 1% of all trials), error events were not explicitly modeled. By virtue of the stimuli sequence design, these regressors were orthogonal to each other so that variance contributing to one stimulus type was not correlated to another stimulus's regressor, even using the exact behavioral response patterns. This analysis was used to identify responses to different stimulus types separately within

each EPI run. Methods for isolating overlapping fMRI responses to interleaved stimulus types and detailed descriptions of these analysis techniques may be found in previous publications (Clark et al., 2001, 2000). Contrasts were specified that evaluated the effects of target stimuli relative to the nontarget baseline. Because multiple voxels were examined, a correction for multiple comparisons based on the theory of Gaussian fields was employed (Worsley, 1994; Worsley and Friston, 1995).

Random effects models were used to determine the main effect of target, distractor and standard stimuli separately for the ERT and the placebo group. These models evaluated whether each group's brain function reflected the expected hemodynamic response patterns previously observed using this task. These analyses combined contrast images from each run into separate one-sample t-tests for targets, distractors, or standards for each group.

To identify functional differences between the placebo and estrogen groups, we used a combination of fixed and random effects models. Because this was a pilot study that used fMRI to examine the effect of a new low estrogen dose manipulation, we were uncertain as to the magnitude of differences, if any. Fixed effects models were used to determine whether differences existed. Because this type of model is relatively insensitive to variance between subjects, it may overestimate the significance of group differences when in fact those differences are due to only a few subjects in the sample. However, fixed effects models have an advantage in that they are based on a substantially larger number of observations, which dramatically increases the ability to detect differences. In fixed effects analyses, all runs from both groups were entered into a single multiple regression model that used t-test contrasts to determine if activity to targets, distractors, or standards differed between study groups. Evidence for statistical significance of group differences was accepted using criteria that were sensitive to either the amplitude or spatial extent of activation. Brain regions showing a difference between groups are reported if they surpass corrections for searching the whole brain ($P < 0.05$ family-wise error) or if they surpass thresholds for spatial extent. This latter criterion defined significant clusters of voxels having a voxelwise entry P-level of 0.001, a minimum clusterwise P-level of 0.05, and a mini-

Table 1
Characteristics of participants

	Mean (SD) or %	
	E_2	Placebo
Age (years)	76.9 (3.94)	79.0 (3.93)
Age at menopause (years)	52.0 (2.78)	46.7 (6.23)
Handedness		
Right	87.5%	100.0%
Education		
High school graduate	12.5%	0.0%
Some college or grad	87.5%	62.5%
Postgraduate work	0.0%	37.5%
Hysterectomy	12.5%	25%
Geriatric Depression Scale		
Baseline	3.0 (4.28)	3.9 (2.70)
3-year	3.8 (5.70)	4.6 (5.26)
Beck Anxiety Scale		
Baseline	2.0 (2.98)	3.8 (3.65)
3-year	5.5 (5.73)	4.3 (2.87)
Folstein MMSE score		
Baseline	28.8 (1.39)	29.5 (0.53)
3-year	28.8 (1.04)	29.4 (0.53)
PASE		
Baseline	134.7 (52.27)	122.0 (87.09)
3-year	102.2 (50.50)	84.5 (32.86)
Estradiol level (pg/ml)		
Baseline	8.4 (4.32)	9.3 (3.52)
3-year	21.0 (14.23)[a]	7.6 (2.25)
Estrone level (pg/ml)		
Baseline	13.4 (5.33)	14.7 (6.01)
3-year	50.9 (50.73)	13.2 (4.17)

MMSE=Mini-Mental Status Examination; PASE=Physical Activity Scale for the Elderly.

[a] $P < 0.05$ compared with placebo group.

mum cluster size of at least 1 cc. This clusterwise correction is sensitive to the spatial extent of contiguous voxels exceeding what would be expected by chance.

Next, we provided more stringent statistical evidence for our results using random effects analyses of the same data. In the random effects analyses, we directly compared the contrast images for estrogen and placebo groups using two-sample t-tests separately for target, distractor, and standard stimuli contrast maps. Regions of difference were evaluated by significance criteria as defined above. It is reiterated that all reported random-effects levels results achieved statistical significance of at least $P < 0.001$ voxelwise, or met more stringent criteria. Tables and figures describing what brain areas were activated by the task are presented. To confirm and extend the results

of these statistical tests, stimulus-onset, time-locked averages (analogous to event related potentials) were obtained from fitted regression estimates for each participant and averaged across voxels within these regions.

3. Results

3.1. Sample characteristics

The study groups did not differ with regard to age ($t_{14} = -1.080$, $P = 0.298$), education ($\chi^2 = 5.111$, $P = 0.276$), hysterectomy status ($\chi^2 = 0.410$, $P = 0.522$), global cognitive function as measured by the Folstein Mini Mental Status Examination at baseline ($t_{14} = 1.426$, $P = 0.176$) and 3-year follow-up ($t_{14} = 1.557$, $P = 0.143$), depression at baseline ($t_{14} = 0.490$, $P = 0.632$) and 3-year follow-up ($t_{14} = -0.289$, $P = 0.777$), anxiety at baseline ($t_{14} = 1.050$, $P = 0.311$) and 3-year follow-up ($t_{14} = -0.506$, $P = 0.621$), and physical activity level at baseline ($t_{14} = -0.352$, $P = 0.730$) and 3-year follow-up ($t_{14} = -0.793$, $P = 0.442$). One subject in the ERT group was left-handed. We note that education was not recorded in units of years, but rather within a range. This may have prevented detecting a significant difference between the groups. Table 1 presents the means (SD) and proportions for the sample characteristics.

3.2. Behavioral performance

A total of 114 fMRI runs were free of movement artifact and were used for subsequent statistical analyses. A total of $n = 62$ runs were from subjects in the ERT group, while $n = 52$ runs were from subjects on placebo. Of these 114 runs, all but one placebo subject's behavioral data were available for analysis of

Table 2
Behavioral performance

Variable name	Mean (SD)		
	Estrogen	Placebo	P
Target reaction time (ms)	530.4 (47.52)	518.9 (53.61)	NS
Distractor false positives	0.03 (0.06)	0.00 (0.00)	NS
Standard false positives	0.61 (0.45)	0.93 (0.60)	NS

group differences ($n = 110$). Behavioral measures include average response time to targets, number of false positives to distractor stimuli, and number of false positives to standard stimuli. Successful target "hits" were defined as a button press that occurred in a 1-s window between 200 and 1200 ms following the onset of any target stimulus. False positives to other stimuli were determined using the same response window. Data for each subject were averaged to provide one data point per subject for group comparisons. Table 2 presents the means, standard deviations and independent sample t-test results.
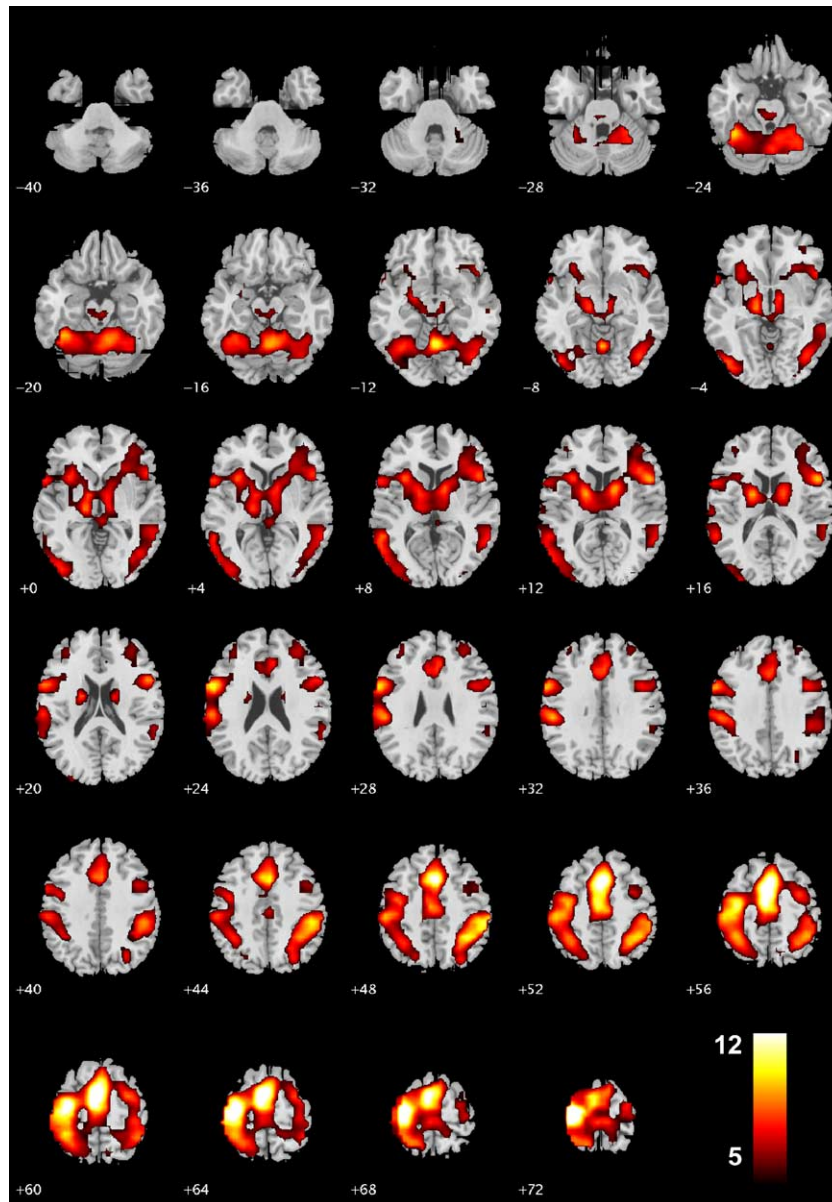

Fig. 1. The illustration shows t-scores for activation to targets for placebo group participants. The image is thresholded at $P < 0.05$ corrected for examining the whole brain. The view is in neurological convention (i.e., left hemisphere is on the left).

Both groups performed equivalently on the behavioral component of the task. There was no significant difference between groups in target reaction time ($t_{14} = -0.445$, $P = 0.664$), number of distractor false positives ($t_{14} = 1.442$, $P = 0.179$), or number of standard false positives ($t_{14} = 1.123$, $P = 0.282$).

3.3. FMRI results

Before analysis of the main effects and group differences on the oddball task, a comparison of runs collected with Z-shimming and non-shimmed EPI sequences was performed using a two-sample t-
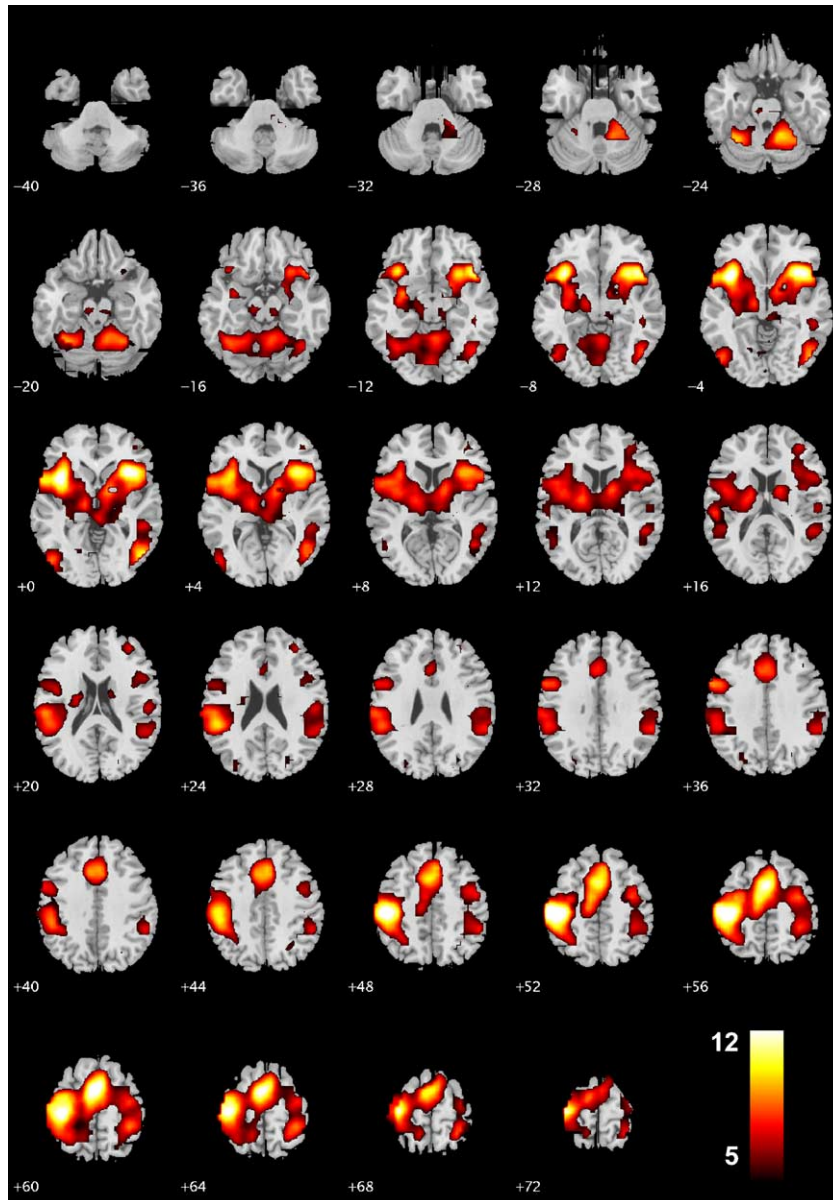

Fig. 2. The illustration shows t-scores for activation to targets for estrogen group participants. The image is thresholded at $P < 0.05$ corrected for examining the whole brain. The view is in neurological convention (i.e., left hemisphere is on the left).

test. Significant differences emerged for Z-shimmed runs in areas consistent with the increased signal-to-noise ratio in ventral brain areas provided by the technique. For target stimuli, Z-shimming showed greater response amplitude in bilateral insula and caudate. Runs without Z-shimming yielded a statistically greater hemodynamic response amplitude to standard stimuli in bilateral precuneus/inferior parietal lobule. These results are consistent with the increased signal-to-noise ratio that would be expected in either the ventral or dorsal aspects of the Z-shimmed and non-Z-shimmed runs, respectively. Chi-square analy-

Table 3
Areas of significant main effects of stimulus presentation for the placebo group

Left hemisphere	Volume (cc)	Right hemisphere	Volume (cc)
Standard stimulus negative signal change[a]			
Cuneus gyrus (17/18/23)	4.608	Cuneus gyrus (7/18/19)	7.584
Lingual gyrus (18/19)	1.344	Lingual gyrus (18/19)	1.344
Posterior cingulate (29/30/31)	2.592	Posterior cingulate (29/30/31)	3.648
Precuneus (7/31)	8.736	Precuneus (7/31)	10.944
		Superior parietal lobule (7)	1.536
Cerebellum (culmen)	2.592	Cerebellum (culmen)	1.152
Distractor stimulus positive signal change[a]			
Middle frontal gyrus (6)	1.152		
Precentral gyrus (4/6)	1.248		
Fusiform gyrus (37)	1.506	Fusiform gyrus (37)	0.960
Middle temporal gyrus (37/39)	2.304	Middle temporal gyrus (37/39)	1.248
Middle occipital gyrus (18)	3.264	Middle occipital gyrus (18/19)	1.536
		Superior occipital gyrus (19/39)	1.440
Inferior parietal lobule (40)	2.880		
Superior parietal lobule (7)	1.536	Superior parietal lobule (7)	1.056
Target stimulus positive signal change[b]			
Thalamus	5.952	Thalamus	5.664
Anterior cingulate (24/32)	6.336	Anterior cingulate (24/32)	4.416
Insula (13)	1.920	Insula (13)	1.632
Inferior frontal gyrus (9/44/47)	5.568	Inferior frontal gyrus (9/44/45/47)	7.008
Medial frontal gyrus (6/8/32)	8.064	Medial frontal gyrus (6/8/9/32)	7.776
Middle frontal gyrus (6/9)	5.760	Middle frontal gyrus (6/9/10/46)	7.680
Paracentral lobule (5/6/31)	2.880	Paracentral lobule (6/31)	1.632
Postcentral gyrus (1/2/3/5/40)	13.152	Postcentral gyrus (1/2/3/40)	7.680
Precentral gyrus (4/6)	12.000	Precentral gyrus (4/6)	5.088
Superior frontal gyrus (6/8)	3.072	Superior frontal gyrus (6/8)	4.320
Fusiform gyrus (19/37)	3.360	Fusiform gyrus (19/37)	2.304
Middle temporal gyrus (37/39)	3.168	Middle temporal gyrus (21/37/39)	2.688
Inferior parietal lobule (40)	7.776	Inferior parietal lobule (40)	7.680
Superior parietal lobule (7)	4.032	Superior parietal lobule (5/7)	3.456
Superior temporal gyrus (13/22/38)	3.936	Superior temporal gyrus (13/22/38)	1.824
		Supramarginal gyrus (40)	1.056
Precuneus	1.536	Precuneus	3.688
Middle occipital gyrus (18/19)	4.224	Middle occipital gyrus	1.248
Globus pallidus	1.334		
		Caudate	1.728
Putamen	4.896	Putamen	2.496
Cerebellum (culmen)	7.968	Cerebellum (culmen)	9.504
Cerebellum (declive)	7.584	Cerebellum (declive)	6.432

[a] Activity in clusters defined by $P < 0.05$ clusterwise-corrected level of significance.
[b] Activity in voxels surpassing $P < 0.05$ corrected for examining the whole brain.

sis confirmed that there was not a different proportion of Z-shimmed and non-Z-shimmed runs in the experimental groups ($\chi^2 = 0.228$, NS). Therefore, all runs were analyzed together to increase statistical power.

Figs. 1 and 2 show target-elicited hemodynamic activity statistical maps from the random effects analyses for each group (placebo and estrogen). Tables 3 and 4 present the anatomical regions having significant hemodynamic activity elicited by target ($P < 0.05$, corrected for searching the whole brain), distractor, or standard stimuli ($P < 0.05$, clusterwise-corrected) that surpass statistical thresholds. All areas

reported as 'active' during the task were supported by evidence from both fixed and random effects tests.

Target stimuli activated widespread brain areas previously observed using this paradigm (Clark, 2002; Clark et al., 2000). Because we used similar clustering thresholds as in past work (i.e., at least 1 cc, or 11 contiguous voxels within an anatomical area), activity to targets in regions not previously reported (i.e., basal ganglia, precuneus, and right superior parietal lobule) may be the result of a larger placebo group sample or slightly different models of hemodynamic response. Also, this analysis observed regions

Table 4
Areas of significant main effects of stimulus presentation for the estrogen group

Left hemisphere	Volume (cc)	Right hemisphere	Volume (cc)
Standard stimulus positive signal change[a]			
Precentral gyrus (4/6)	2.592		
Middle temporal gyrus (37/39)	1.056		
Standard stimulus negative signal change[a]			
Cuneus gyrus (17/18)	1.440		
Precuneus (7/31)	4.992	Precuneus (7/31)	4.608
Distractor stimulus positive signal change[a]			
		Cuneus	0.960
Target stimulus positive signal change[b]			
Thalamus	6.114	Thalamus	7.008
Anterior cingulate (24/32)	5.280	Anterior cingulate (24/32)	3.840
Insula (13)	7.008	Insula (13)	4.800
Inferior frontal gyrus (9/44/47)	6.240	Inferior frontal gyrus (44/45/47)	6.636
Medial frontal gyrus (6/8/32)	7.872	Medial frontal gyrus (6/8/9/32)	6.240
Middle frontal gyrus (6/9)	3.360	Middle frontal gyrus (6/10)	5.568
Paracentral lobule (5/6/31)	1.920		
Postcentral gyrus (1/2/3/40)	10.944	Postcentral gyrus (1/2/3/40)	5.856
Precentral gyrus (4/6)	2.688	Precentral gyrus (4/6)	5.952
Superior frontal gyrus (6/8)	2.208	Superior frontal gyrus (6/8)	2.784
Fusiform gyrus (19/37)	1.920	Fusiform gyrus (19/37)	1.440
Middle temporal gyrus (37/39)	1.536	Middle temporal gyrus (21/37/39)	6.720
Inferior parietal lobule (40)	8.352	Inferior parietal lobule (40)	2.112
Superior parietal lobule (7)	3.936	Superior parietal lobule (5/7)	2.112
Superior temporal gyrus (13/22/38)	1.536	Superior temporal gyrus (13/22/38)	3.168
Supramarginal gyrus (40)	7.488	Supramarginal gyrus (40)	1.056
Lingual gyrus (18)	2.688	Lingual gyrus (18)	1.440
Middle occipital gyrus (18/19)	1.440		
Globus pallidus	2.112	Globus pallidus	2.016
Putamen	5.856	Putamen	5.664
		Amygdala	1.152
Cerebellum (culmen)	8.848	Cerebellum (culmen)	7.008
Cerebellum (declive)	5.088	Cerebellum (declive)	5.760

[a] Activity in clusters defined by $P < 0.05$ clusterwise-corrected level of significance.
[b] Activity in voxels surpassing $P < 0.05$ corrected for examining the whole brain.

of positive signal change to distractors in several areas of prefrontal, temporal, parietal and occipital cortex. Although no clusters of positive BOLD signal change surpassed statistical thresholds for standard stimuli in the placebo group, areas of precentral gyrus and middle temporal gyrus activation to standards were detected in the estrogen group. There was a negative BOLD signal change in response to standard stimuli for both estrogen and placebo group participants in medial areas of posterior cortex. In placebo participants, such activity was observed in occipital lobe, precuneus, posterior cingulate and cerebellum. On estrogen one-sample t-test maps, this 'deactivation' appears limited to occipital lobe. Inspection of group maps showed other possible differences in main effects. To distractor stimuli, placebo participants activated a network of left prefrontal and bilateral temporal-occipital brain areas, whereas hemodynamic

Table 5
Statistically significant regions of difference where placebo participants have greater hemodynamic activity than estrogen participants

Cluster maximum t-score coordinates							
Gray matter regions	Brodmann areas	x	y	z	Cluster cc	Cluster avg t-score	Cluster max t-score
Targets							
Cluster 1		−16	−72	60	23.712	4.39**	7.67***
Left angular gyrus	39						
Left inferior parietal lobule	39,40						
Left middle occipital gyrus	18,19						
Left middle temporal gyrus	19,39						
Left precuneus (PCu)	19,7						
Left superior parietal lobule	40,7						
Cluster 2		16	0	16	17.664	4.53**	8.05***
Bilateral caudate/putamen							
Left inferior frontal gyrus	11,47						
Left middle frontal gyrus	11						
Cluster 3		−60	−52	−6	9.312	4.41**	7.36***
Left fusiform gyrus	37						
Left inferior temporal gyrus	19,20,37						
Left middle temporal gyrus	21,37						
Left superior temporal gyrus	22						
Cluster 4		36	−68	54	4.896	4.10*	5.50***
Right inferior parietal lobule	40						
Right precuneus (PCu)	19						
Right superior parietal lobule	7						
Distractors							
Cluster 1		−48	−80	12	13.536	3.78*	5.81***
Left middle occipital gyrus	18,19						
Left middle temporal gyrus	21,37,39						
Left superior temporal gyrus	22,41,42						
Left transverse temporal gyrus	41						
Cluster 2		16	−4	−6	7.968	3.58	4.72**
Left inferior frontal gyrus	11,47						
Left middle frontal gyrus	11						
Bilateral caudate/putamen							
Cluster 3		−52	−52	48	4.992	3.70	5.15***
Left inferior parietal lobule	40						
Left superior parietal lobule	7						
Cluster 4		−20	−72	54	3.840	3.90*	5.79***
Left precuneus (PCu)	19,7						
Left superior parietal lobule	7						

Reported clusters at $P<0.05$ clusterwise significance level, corrected for multiple comparisons, comprising at least 1 ml of tissue. *$P \leq 0.0001$; **$P \leq 0.00001$; ***$P \leq 0.000001$, voxelwise significance level.

activity surpassed statistical thresholds in only a small occipital cortex area in estrogen participants. There were no obvious differences in the target activation maps.

Differences between the groups in brain activity that occurred to target, distractor, or standard stimuli were first evaluated using a fixed-effect regression model. For targets, the fixed effects model identified regions of significantly greater activity in estrogen compared with placebo participants for all three stimuli classes. For targets, estrogen participants showed greater hemodynamic response in right occipital

cuneus/precuneus and left postcentral gyrus. For distractors, a region encompassing right anterior cingulate/medial frontal gyrus and left medial frontal gyrus/superior frontal gyrus had greater activity in estrogen participants. For standard stimuli processing, estrogen participants showed greater activity in bilateral posterior cingulate/precuneus and an area in left cerebellum. It is important to note that these areas were associated with negative BOLD signal change when each group was examined separately, indicating that this difference represents differences in degree of 'deactivation' to standards. There also were regions in which hemo-

Table 6
Statistically significant regions of difference where estrogen participants have greater hemodynamic activity than placebo participants

Cluster maximum *t*-score coordinates							
Gray matter regions	Brodmann areas	x	y	z	Cluster cc	Cluster avg *t*-score	Cluster max *t*-score
Targets							
Cluster 1		20	−84	30	6.114	3.57	4.47**
Right cuneus	18						
Right precuneus (PCu)	19						
Cluster 2		−56	−16	48	4.896	4.19*	6.27***
Left inferior parietal lobule	40						
Left postcentral gyrus	1,2,3,40						
Left precentral gyrus	4,6						
Distractors							
Cluster 1		−12	44	36	12.000	3.58	4.67**
Left medial frontal gyrus	9						
Left middle frontal gyrus	10						
Left superior frontal gyrus	10,9						
Right anterior cingulum	32						
Right medial frontal gyrus	10,9						
Right superior frontal gyrus	9						
Standards							
Cluster 1		20	−84	42	60.768	3.96*	6.856***
Bilateral thalamus							
Bilateral cuneus	18,19						
Bilaeral lingual gyrus	18,19						
Bilateral parahippocampal gyrus	30						
Bilateral posterior cingulate gyrus	31						
Bilateral posterior cingulum	23,29,30,31						
Bilateral precuneus (PCu)	23,31,7						
Right superior parietal lobule	7						
Cluster 2		20	44	30	4.416	3.77*	4.84***
Right superior frontal gyrus	9,10						
Cluster 3		−56	−64	12	3.264	3.43	4.22*
Left middle temporal gyrus	19,7						
Left superior temporal gyrus	19,7						
Left supramarginal gyrus	7						

Reported clusters at $P<0.05$ clusterwise significance level, corrected for multiple comparisons, comprising at least 1 ml of tissue.
*$P\leq0.0001$; **$P\leq0.00001$; ***$P\leq0.000001$, voxelwise significance level.
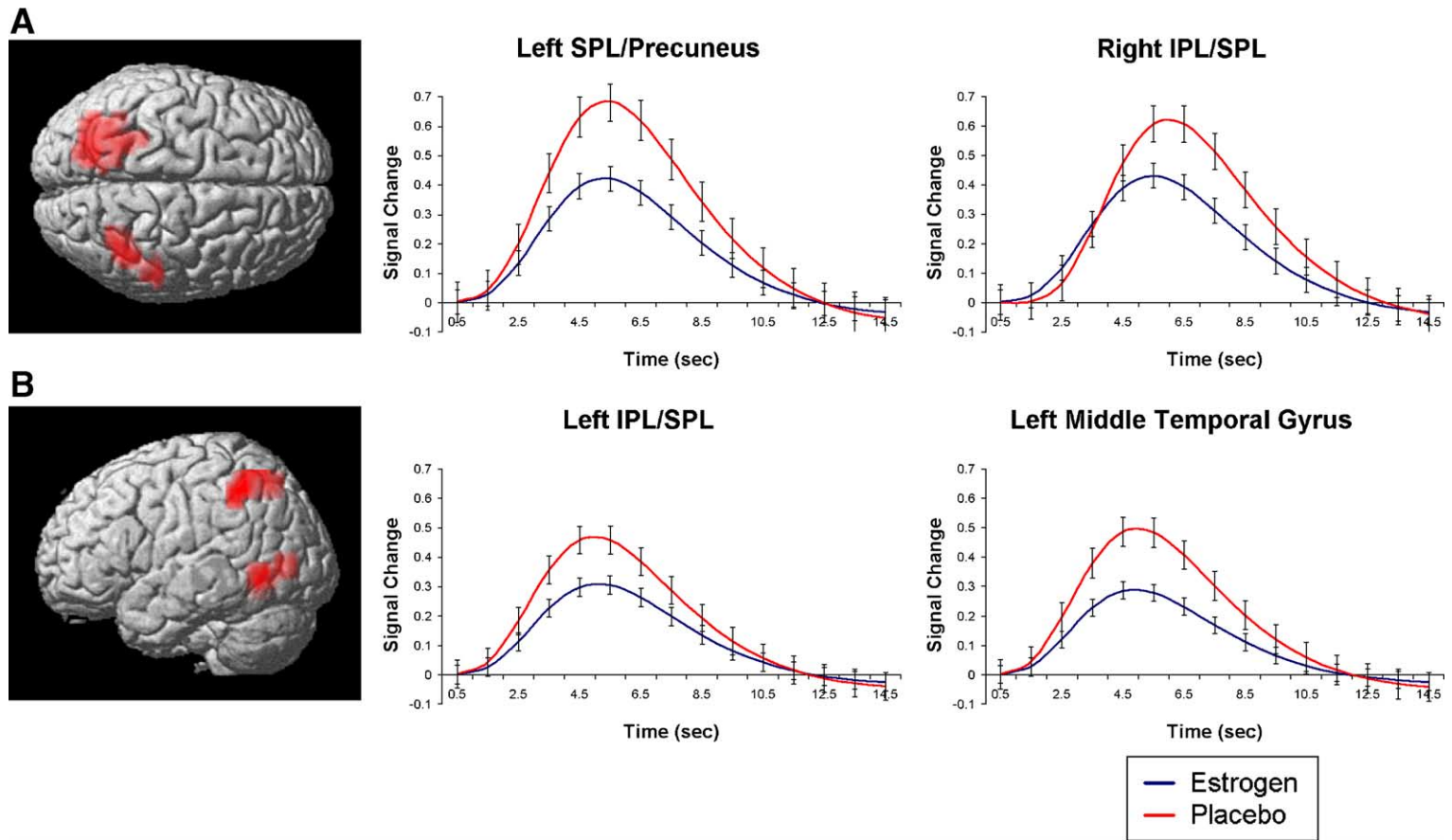
Fig. 3. (A) Area of left inferior parietal lobule (IPL) and right superior parietal lobule (SPL)/IPL in which estrogen participants show significantly less negative BOLD signal change compared with placebo participants in response to target stimuli. (B) Area of left IPL and left middle temporal gyrus in which estrogen participants show significantly less negative BOLD signal change compared with placebo participants in response to distractor stimuli. All clusters of activation are significant at $P < 0.05$, clusterwise-corrected for multiple comparisons.

dynamic activity was greater in placebo participants compared with those on ERT. For targets, placebo subjects showed greater BOLD response in several left hemisphere areas, including superior/inferior parietal lobule, middle occipital gyrus/middle temporal gyrus (BA 39) and another area of middle temporal gyrus (BA 21/22/37). Placebo subjects showed greater hemodynamic activity to distractor stimuli in several areas. In the left hemisphere, one cluster of difference comprised occipital-temporal regions while other was localized to inferior parietal lobule (BA 40). There also were clusters of significantly different brain activity in basal ganglia grey matter areas and left precuneus (BA 7). One final area where distractors elicited greater brain activity in placebo subjects was seen in left middle/inferior frontal gyrus; however, this was at a trend level of significance (*P* < 0.10, clusterwise-corrected). There were no areas of greater hemodynamic activity to standard stimuli in placebo subjects compared with estrogen subjects. Tables 5 and 6 present the results of the fixed effects model evaluating ERT and placebo group differences. It is noted that possible activity differences between groups in the cerebellum were not examined because not all participants' activity in this sample had sufficient MR slice coverage to fully characterize cerebellum function.

More stringent evidence for the validity of these group differences was obtained by reexamining the data using a random effects analysis. In this approach, statistical differences in brain function between experimental groups were tested using two-sample *t*-tests separately for distractor, target, and standard (deactivation) contrast maps. As described above, several measures were used to determine statistical significance of the obtained results. First, a voxelwise *P*-level of 0.001 was used to select voxels for consideration of cluster membership. Only clusters that surpassed a clusterwise *P* < 0.05 corrected for multiple comparisons level of significance and comprised at least 1 cc of grey matter were judged statistically significant. Fig. 3A and B display these areas. Fig. 3A shows target activity differences in hemodynamic response amplitude between placebo and estrogen participants. Placebo subjects had higher activity in a cluster comprising areas of left superior temporal lobule and a cluster that included right superior temporal lobule and right inferior parietal lobule. Fig. 3B shows greater hemodynamic response amplitude to distractor stimuli in placebo compared with estrogen participants in a cluster of left middle temporal gyrus and another cluster of left superior parietal lobule/inferior parietal lobule. Fig. 3A and B also show the fitted hemodynamic response estimates of the BOLD time course averaged across voxels within each region of difference. Random effects analysis of group differences to standard stimuli confirm that these differences reflect relatively less negative BOLD signal change for estrogen participants compared with placebo participants (Fig. 4). Furthermore, the results suggest that this effect may be best localized to a cluster comprising 1.152 cc of left posterior cingulate and 1.728 cc of right posterior cingulate.

Random effects analysis did not find group differences in any frontal or subcortical brain area observed in the fixed effects analysis. It also should be emphasized that none of our group comparisons found differences in which brain structures were active during task processing, only differences in amplitude of response in those regions known to be activated by the task.

Because the effects of handedness on brain activity involved in cognitive and motor processes are not
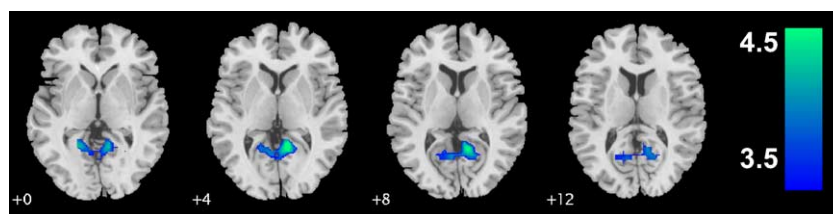

Fig. 4. The illustration shows *t*-scores for activation to standard stimuli for the estrogen group relative to placebo. Areas of posterior cingulate demonstrate significantly less negative BOLD signal change compared with placebo participants. The cluster is significant at *P* < 0.05 clusterwise-corrected for multiple comparisons. The view is in neurological convention (i.e., left hemisphere is on the left).

completely understood, these analyses were redone with the omission of the one left-handed person in the sample. As expected, the results were the same.

4. Discussion

These analyses explored the effects of ultralow dose micronized estradiol (0.25 mg/day) therapy on hemodynamic activity associated with selective attention and automatic orienting cognitive processes in a group of older women. This study found hemodynamic activity differences between groups of women on ERT and those on placebo. For target stimuli, ERT subjects showed reduced activity in bilateral parietal lobe cortex. For distractor stimuli, differences in brain function were lateralized to left hemisphere parietal and temporal structures. In both instances, estrogen significantly reduced the amplitude of hemodynamic activity in these areas. Insofar as increased hemodynamic amplitude indicates sustained neural activity associated with increased mental effort (Just et al., 1996; Raichle et al., 1994), these results suggest that ERT reduces the need for sustained information processing in posterior brain areas known to be involved in attention, specifically motor attention (Astafiev et al., 2003; Rushworth et al., 2003). These results are consistent with a study that used a two-tone auditory oddball task to examine the effects of estrogen and progestogen on the P300 event-related potential (Anderer et al., 2003). This study found that estrogen alone did not affect P300 amplitude, but it significantly reduced P300 latency, suggesting more efficient processing. Similarly, visual inspection of the fitted hemodynamic response curves notes reduced latency to peak target hemodynamic amplitude in the ERT group. Although the relationship of electrophysiological and hemodynamic measures is not yet fully understood, this similarity bears further scrutiny. Because there were no behavioral differences on the auditory oddball task, it is not possible to directly link these ERT-related neural changes to behavior differences. Future studies could more fully explore the meaning of these effects by including a detailed neuropsychological battery or by using multiple neuroimaging measures to assess different cognitive functions.

This study also found evidence that low-dose ERT may increase hemodynamic response to both target and distractor stimuli in prefrontal brain areas. This finding needs to be replicated with a larger sample employing effective control over between-subjects variance. However, the overall pattern of findings is reasonable within the context of changes in top–down mediated control of posterior brain function by prefrontal lobe function, as been previously found in an electrophysiological human lesion study (Barcelo et al., 2000). The current findings also are consistent with previous ERT neuroimaging studies, which generally show increases in prefrontal activity accompanied by either increases or decreases in posterior cortex activity depending on task demands.

For both placebo and estrogen groups, there are areas of negative BOLD signal change in posterior cortex to frequently presented standard stimuli. Task-related 'deactivation' areas include primary occipital cortex, precuneus, posterior cingulate and cerebellum. Such BOLD signal reductions have been interpreted as resource re-allocation (McKiernan et al., 2003), likely serving to better utilize attention resources for target identification. ERT appears to have altered the degree of BOLD signal reduction observed during standard stimulus processing. Women taking estradiol had relatively less negative signal change in bilateral posterior cingulate. Given the proximity to posterior ventricles, this may simply reflect differences in susceptibility artifact between the two groups secondary to group differences in ventricular size. However, this more likely represents a real effect of ERT because the differences occur in brain areas known to have negative BOLD signal change and are consistent with previous studies of task-induced deactivation. McKiernan et al. (2003) found BOLD reductions in bilateral posterior cingulate to correspond with increases in attentional task difficulty. In particular, the left posterior cingulate 'deactivated' when stimulus discriminability was harder, while right posterior cingulate 'deactivated' following increases in short-term memory load. These previous findings are consistent with the interpretation that these ERT-related BOLD response differences may be associated with greater cognitive processing efficiency. It also is notable that the posterior cingulate is involved in both emotion and learning/memory processing (Maddock et al., 2003). The finding that estrogen may modulate

activity in this structure suggests a possible locus for the effect of estrogen's role in reducing negative mood symptoms during menopause.

Despite a sizable electrophysiological literature exploring brain function in older persons on odd-ball-type tasks, this is the first fMRI experiment using the visual oddball task to examine hemodynamic activity in older adults. Targets elicit the same brain regions in older women as observed in previous visual fMRI oddball studies of younger adults (Clark et al., 2000; Kiehl et al., 2001) despite an approximately 75 ms slower target reaction time compared with behavioral data obtained in younger adults (Clark et al., 2000). Unfortunately, because target stimuli activate such a widespread network of brain structures, a visual inspection of target-elicited activity in the placebo group is insufficient to easily identify differences from our previous results. However, there are some noteworthy visual comparisons for hemodynamic activity elicited by other oddball stimuli. Distractors showed activity in a network of left prefrontal and bilateral temporal-occipital brain areas in older placebo subjects that was only observed in one (Clark, 2002) of two (Clark, 2002; Clark et al., 2000) previous reports of younger adult data using this paradigm. This suggests there may be differences in neural activity underlying automatic orienting to distractor stimuli. Such differences are consistent with several electrophysiological studies of normal aging using three-stimulus oddball tasks (Friedman and Simpson, 1994). These studies show that target- and distractor-elicited P300 latencies begin to increase with age (Polich, 1997), with appreciable increases in the rate of latency slowing appearing after age 45–50 (Brown et al., 1983). Large samples show slowing of target-elicited P300 latency across all scalp sites with increasing age (Anderer et al., 1998), but slowing is best detected at centro-parietal (Cz and Pz) sites (Coyle et al., 1991). Topographically, there is some evidence that the task-relevant (i.e., target-elicited) P3b component shifts from a parietal to a more frontal maximum with aging (Friedman et al., 1997, 1993; Friedman and Simpson, 1994).

It is acknowledged that the use of fMRI BOLD imaging as a tool for aging is still undergoing evaluation. D'Esposito et al. (2003) review evidence suggesting that altered neurovascular coupling in older persons may be the result of cerebrovascular pathol-

ogy or normal age-related changes. When such potential confounds exist, it limits the ability to attribute BOLD signal changes to alterations of neural activity in older samples (D'Esposito et al., 2003). It currently is not known whether these types of confounds also might be imposed by ERT treatment itself. Several studies using different imaging modalities have examined neural activity in samples of women undergoing ERT. However, no studies have attempted to use blood flow quantification measures such as MR perfusion to determine whether estrogen's vasodilative properties confound hemodynamic measurement. Moreover, no study has yet attempted to empirically link ERT-induced changes in brain function to any of the specific mechanisms proposed to mediate its effect. Several mechanisms have been examined, including direct neurotransmission effects on ER-α and ER-β receptors that are differentially located throughout the brain, or effects on second messenger systems through receptors located on dendrites, presynaptic terminals and glial cells (McEwen, 2001). Currently, the exact mechanism of ERT's neuromodulatory effect is not known.

Several other factors could have influenced the results of this study. There are commonly observed differences in general health, education, and motivation between women who take ERT and those who do not. This raises the possibility that placebo and ERT groups may differ on characteristics that would influence brain function. The current study, which was a double-blind longitudinal study, likely circumvented this problem, as both groups of volunteers were highly motivated. Future studies of ERT effects on brain activity will ideally employ a double-blind cross-over design, as has been applied in other studies (Shaywitz et al., 1999) and which is the optimal solution for this problem. Furthermore, all subjects were in good health, had normal general intellectual ability, and were equivalent in self-reported depression and anxiety. The results were not influenced by the one left-handed person in the sample. When the data were re-analyzed without that subject, the results did not change. Finally, previous studies have suggested that long-term ERT may have cumulative neuroprotective effects (Garcia-Segura et al., 2001; Maki and Resnick, 2000). Because fMRI data were not available at the beginning of the study, these data cannot address whether brain-activity differences

were influenced by cumulative neuroprotective effects, or whether the effects seen may be the product of existing baseline differences between groups.

The results of the recent Women's Health Initiative Memory Study (WHIMS) strongly suggested a harmful effect of combined conjugated equine estrogen plus progestin with respect to risk for Alzheimer's disease or cardiovascular health. However, several important questions remain unanswered. First, the current results show that ultra-low doses of ERT result in measurable changes to neural activity that appear to be beneficial to neural function. It is possible that the negative health risks identified in the WHIMS may be the result of dose, duration of treatment, or another mechanism not yet identified. Because the exact mechanism by which conjugated equine estrogen plus progestin exerts a detrimental effect is unknown, it is possible that ERT may only impose risk to a subgroup of persons. However, it is acknowledged that initial efforts to examine the WHIMS sample for such subgroups have not yet yielded results (Rapp et al., 2003). Second, because the WHIMS aimed to evaluate the relationship between ERT and dementia risk, the majority of women in the WHIMS study did not meet criteria for detailed neuropsychological assessment (i.e., they must have had a Mini-Mental Status Examination score <80) (Shumaker et al., 2003). Therefore, the WHIMS did not evaluate possible advantageous effects of ERT in women who did not subsequently develop cognitive problems. It is possible that ERT might have conveyed substantial cognitive benefits in the absence of health risk that might have been detected through detailed cognitive assessment or neuroimaging. Results that could speak to this possibility have not yet been reported from other large scale studies such as the Women's Health Initiative Study of Cognitive Aging (WHISCA) and the cognitive component of the canceled Women's International Study of Long Duration Oestrogen after Menopause-Cognition (WISDOM-COG).

In conclusion, the present study adds to a handful of other neuroimaging studies of pre-, peri-, and post-menopausal women indicating that estrogen alters brain activity. In order to characterize estrogen's effects and mechanisms of action, future research should consider a systematic examination of how factors like task difficulty and ERT dose affect brain activity on various different tasks that elicit hemodynamic activity in neural circuitry. Such research will help us to better understand and isolate the mechanism of estrogen's beneficial effects, so that a more informed judgment can be made regarding the risks and benefits to patients undergoing ERT.

Acknowledgments

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