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Estrogen enhances hippocampal gray matter volume in young and older postmenopausal women: A prospective dose response study

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Abstract

Estrogen administration following menopause has been shown to support hippocampally-mediated cognitive processes. A number of previous studies have examined the effect of estrogen on hippocampal structure to determine the mechanism underlying estrogen effects on hippocampal function. However, these studies have been largely observational and provided inconsistent results. We examined the effect of short-term estradiol administration on hippocampal gray matter volume in a prospective study with multiple doses of estradiol (placebo, 1 mg, and 2 mg). Following three months of estradiol administration bilateral posterior hippocampal voxel-based gray matter volume was increased in women who received 2 mg estradiol. There were no significant differences in total hippocampal volume and no significant effects on gray matter volume in women who received placebo or 1 mg estradiol. These findings accord with previous animal studies and provide evidence of estrogen effects on hippocampal morphology that may represent a neurobiological mechanism for estrogen effects on cognition in post-menopausal women.

1. Introduction

Cognitive changes during the menopause-transition and following menopause in women are common (Halbreich et al., 1995; Weber et al., 2014; Woods et al., 2000). Reported changes during menopause include reductions in both delayed and immediate recall (Epperson et al., 2013) and minor decreases in concentration and processing speed (Kok et al., 2006). Additionally, cognitive changes in post-menopausal women are greater than expected from the effects of age alone (Halbreich et al., 1995), suggesting that the decline in estrogen during menopause may have negative consequences for cognitive performance (for review

see: Maki and Dumas, 2009; Newhouse and Dumas, 2015; Sherwin and Henry, 2008). Past studies have also consistently observed cognitive decline and increased risk for dementia in women who undergo early menopause, thus experiencing a greater period of life with low ovarian hormone levels (Farrag et al., 2002; Nappi et al., 1999; Rocca et al., 2007). This further supports a negative impact of estrogen loss beyond the effects of age in older women.

The cognitive benefit of estrogen may be mediated in part by estrogen effects on the hippocampus. The hippocampus is implicated in memory function and has been shown to have an abundance of estrogen receptors (for review see Bean et al., 2014; Hara et al., 2015; Österlund et al., 2000; Woolley, 1998), suggesting that it may be a structure that is particularly sensitive to the effects of estrogen. Previous studies support that hormone replacement with estrogen has beneficial cognitive effects in postmenopausal women. In observational studies long-term hormone replacement is associated with better performance in a range of cognitive domains, including working and episodic memory, (Berent-Spillson et al., 2010; Maki et al., 2011; Phillips and Sherwin, 1992; Sherwin, 1997). Additionally, experimental administration of estrogen benefits hippocampally-mediated cognitive processes (Daniel et al., 2015; Jacobs et al., 1998; Verghese et al., 2000).

These benefits of estrogen on hippocampally-mediated cognitive processes may parallel positive effects of estrogen on hippocampal structure. Animal studies demonstrate that estrogen administration is associated with increased hippocampal dendritic density (Lewis et al., 1995; Woolley, 1998; Woolley and McEwen, 1992). The observed increases in dendritic density following estrogen administration is blocked by estrogen receptor antagonists (Lewis et al., 1995), supporting that estrogen has direct effects on morphology.

Previous human studies examining morphology changes with ovarian hormone replacement in postmenopausal women have reported inconclusive or conflicting results (Wnuk et al., 2012). Cross-sectional studies report greater hippocampal volume in women who previously received hormone replacement compared with never users (Boccardi et al., 2006; Lord et al., 2008), although others found no difference in hippocampal volume between past users and non-users of hormone replacement (Resnick et al., 2009). These studies are limited by being observational rather than prospectively administering estrogen or have included combined estrogen and progesterone hormone regimens that may have reduced effects compared with estrogen alone (Maki et al., 2007). Additionally, previous studies have not generally used voxel-based methods to assess changes in gray matter volume which may be more sensitive to the effects of short-term estrogen in the hippocampus than region of interest total volume measures (Mechelli et al., 2005).

The purpose of this study was to determine whether estradiol (E2) administration has an acute effect on hippocampal volume in post- menopausal women. Women completed an MRI scan before and after 3 months of receiving 17 β -estradiol or placebo. We hypothesized that administration of E2 would result in greater increases in hippocampal gray matter volume than in women receiving placebo.

2. Methods

2.1. Participants

Seventy-five postmenopausal women were included in this study. Participants were recruited for two studies: one examining the effects of E2 administration on cognitive function (Dumas et al., 2013; Vega et al., 2016) and the other examining the effect of E2 administration on the stress response (unpublished). Participants completed the study at either Vanderbilt University Medical center or the University of Vermont. These studies were approved by the Vanderbilt University Medical Center or University of Vermont Institutional Review Boards and informed consent was obtained from all participants.

2.2. Cognitive Screening

All participants were cognitively assessed using the Mini Mental State Exam (MMSE) (Folstein et al., 1975), Brief Cognitive Rating Scale, and the Mattis Dementia Rating Scale (DRS-2) to establish a Global Deterioration Scale score (GDS) (Reisberg et al., 1982). Participants were required to have a GDS score of 1-2 and a MMSE score of greater than 26. No participant scored below 123 on the Mattis scale (Table 1).

2.3. Estradiol Administration

All participants were postmenopausal, without menses for at least 1 year with FSH level greater than 27 mIU/ml. Women with bilateral oophorectomy were excluded from participation. None of the participants were taking ovarian hormones, hormone therapy, or hormonal contraception at enrollment and were at least one year without such treatment. Participants were physically healthy with a body mass index ≤ 33 kg/m². Participants with major concomitant illnesses were excluded for abnormal findings on the basis of history, physical exam (including EKG), and laboratory tests assessing hematopoietic, renal, hepatic and hormonal function (complete blood count, serum chemistries, thyroid stimulating hormone, urinalysis). Participants were required to have had a normal mammogram within the last year.

Participants were excluded if they had specific contraindications for E2 treatment. Specific criteria for exclusion for the E2 treatment included contraindications for hormone replacement including history of breast cancer or E2-dependent neoplasia; blood pressure $> 160/100$ (untreated); history of deep vein thrombosis or other thromboembolic disease; hepatoma; severe migraines or stroke on oral contraceptives; diabetes; untreated thyroid disease; clinical osteoporosis; and severe menopausal symptoms. Menopausal symptoms were measured using a 60 item (somatic including vasomotor symptoms and mood symptoms) self-reported Menopause Symptoms Checklist. Each item on the checklist was rated from 0 (not at all) to 4 (extremely) for how much each symptom had bothered that participant in the last month, with a possible maximum total score of 240. All participants had a score below 50 on the Menopause Symptoms Checklist (Table 1).

Participants in the first study received double-blinded 1 mg oral 17 β -estradiol or placebo daily for 3 months. Participants in the second study received open-label 1 mg oral 17 β -

estradiol daily for 1 month and then 2 mg oral 17 β -estradiol daily for 2 months to limit side effects during treatment.

2.4. Imaging Procedures

Participants at both sites were scanned on a Philips 3.0 Tesla Achieva scanner, with an 8 channel head coil. Participants had repeated MRI sessions: once at enrollment before beginning E2 or placebo administration, following 3 months of treatment. All participants received the following MR sequences at each MRI session:

1. Sagittal T1-weighted 3D Turbo Field Echo Sensitivity Encoding (TFE SENSE) sequence perpendicular to the anterior commissure (AC) -posterior commissure (PC) line, repetition time (TR) of 9.9 ms, echo time (TE) of 4.6 ms, a flip angle of 8°, number signal averages (NSA) 1.0, a field of view (FOV) of 256 mm, a 256 \times 256 matrix, and 1.0 mm slice thickness with no gap for 140 contiguous slices.
2. T2- weighted Gradient and Spin Echo (GRASE) sequence parallel to the AC-PC line, TR 2470 ms, TE 80 ms, NSA 3.0, FOV of 230 mm, 0.7 mm slice thickness with 5.0 mm gap for 28 slices

2.5. Hippocampal Gray Matter Volume Assessment

Morphological changes in the hippocampus were assessed by two methods: a region of interest (ROI) approach and modulated voxel-based morphometry (mVBM). The ROI-based method provides a measure of total hippocampal gray matter volume change with treatment while the mVBM approach allows for the assessment of gray matter volume change by voxel and the identification of clusters of voxels within the hippocampus that show changes in gray matter volume with treatment.

ROI Hippocampal Gray Matter Volume—ROI Hippocampal volume analysis was performed using the FreeSurfer image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>), including motion correction and averaging, removal of non-brain tissue, segmentation of the subcortical white matter and deep gray matter volumetric structures, automated topology correction and surface deformation. The FreeSurfer longitudinal stream (Reuter et al., 2012) was used to create an unbiased within participant template. This template was then used to run the standard “recon-all” procedure with default settings. Each scan was inspected to identify any areas where non-brain was included or brain was excluded. Manual corrections to the brain mask were made as appropriate and the recon procedure was run again with the corrected mask. The hippocampus segmentations were specifically inspected for errors but no manual corrections were required. We then analyzed the volume change of right and left hippocampal volume between time points (pre and post treatment). Hippocampal volumes were normalized by the estimated intracranial volume to account for variation in head size.

mVBM Hippocampal Gray Matter Volume—For each subject, a within subject average image was first created using the pairwise longitudinal registration function in SPM12. This method creates an unbiased average image along with a jacobian distance image. Each average T1 was segmented using the standard processes in SPM12. The gray matter tissue

image for the average T1 was then multiplied by the jacobian distance image to create a gray matter change image for each subject. In this image, negative values show a decrease in gray matter volume from pre-treatment to post treatment, while positive values show increase from pre-treatment to time post treatment.

We then used the DARTEL tools in SPM12 to create a study-specific template, warps for each subject to this template, and the transform of the template to MNI space. The warps and transform were then applied to the gray matter change images to create images in MNI space for comparison. 2nd level analysis was conducted using SPM12 to identify clusters within the hippocampus (anatomical ROI from the human AAL atlas defined in WFU PickAtlas) where gray matter volume showed a significant change with E2 dose (with cluster threshold correction for multiple comparisons: uncorrected $p = 0.05$, $k = 53$, corrected $p < 0.05$ voxel-based $p = 0.001$, $T = 1.73$). mVBM gray matter volume change values (pre to post treatment change in signal per voxel) within these clusters were extracted.

2.6. Statistical Analysis

Univariate ANCOVAs were conducted for E2 dose (placebo, 1 mg E2, 2 mg E2) effect on right and left hippocampal volume change (pre to post 17 β -estradiol change in signal per voxel) and mVBM gray matter volume change within the clusters identified in the 2nd level analysis for E2 dose effects using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Age and intracranial volume were included as covariates in both ROI and mVBM analyses.

This study was conducted at two sites; the 0 and 1 mg groups had participants both at the University of Vermont and Vanderbilt University Medical Center while all of the 2 mg group participants completed the study at Vanderbilt University Medical Center. To assess a possible effect of study site, independent samples t-tests for baseline volume and mVBM measures were conducted. There were no significant differences in the baseline right ($t(73) = 0.07$, $p = 0.95$) or left ($t(73) = 0.11$, $p = 0.92$) hippocampal volumes or right ($t(73) = 1.08$, $p = 0.28$) and left ($t(73) = 0.89$, $p = 0.38$) cluster mVBM gray matter volume between study sites.

To address a possible relationship between E2 dose and age (due to differences in included age range in the studies) a univariate ANOVA examining mean age in the three E2 dose groups was conducted. There was a significant difference in age between the E2 dose groups ($F(2, 72) = 12.58$, $p < 0.001$). The 2 mg study included a wider age range in inclusion criteria so that the group that received 2 mg E2 had a significantly older mean than the 1 mg E2 and the placebo E2 group (Table 1). There was no significant difference in age between the 1 mg and placebo E2 groups ($t(39) = 1.61$, $p = 0.12$).

Secondary analyses were conducted including only participants 60 years of age and younger (the age range included in the placebo and 1 mg study) which brought the 2 mg group to $n = 15$.

3. Results

Participants were 75 postmenopausal women between the ages of 51 to 75, $M=58.19$ ($SD=5.42$). Women were treated with placebo ($n=21$), 1 mg ($n=21$), or 2 mg ($n=33$) E2 for 3 months.

In the age-restricted (60 and younger) analysis a univariate ANOVA did not show a significant relationship between age and dose ($F(2, 54) = 1.41$, $p = 0.25$). Subsequent analyses were conducted including all participants and on an age-restricted subset of participants.

3.1. ROI Hippocampal Gray Matter Volume

Univariate ANCOVAs examining the effect of E2 dose in right and left hippocampal volume change (controlling for intracranial volume and age) showed no significant effect of E2 dose on hippocampal volume (right: $F(2,70) = 1.36$, $p = 0.26$; left: $F(2,70) = 0.29$, $p = 0.75$). There were no significant effects of intracranial volume or age. Restricting the analysis to participants less than 60 years of age did not result in any significant results in these analyses.

3.2. mVBM Hippocampal Gray Matter Volume

Linear regression analyses of mVBM hippocampal gray matter volume change (controlling for intracranial volume and age) demonstrated significant positive effects of E2 dose in bilateral posterior hippocampus clusters (right MNI: 30, -4, 3; Left MNI: -35, -39, -3) (Figure 1). There were no clusters that showed significant negative effects of E2 dose.

In univariate ANCOVAs including all participants, there was a positive effect of E2 dose on mVBM gray matter volume change in both right ($F(2,71) = 4.69$, $p < 0.012$) and left ($F(2,71) = 3.82$, $p < 0.027$) posterior hippocampal clusters. Post hoc Tukey tests showed a significantly greater change in mVBM gray matter volume in the 2 mg group (right: $M = 0.0013$, $SD = 0.0018$; left: $M = 0.0017$, $SD = 0.0029$) compared to placebo (right: $M = -0.0002$, $SD = 0.0022$; left: $M = -0.0002$, $SD = 0.0026$) (right: $p = 0.037$, left: $p = 0.030$) and 1 mg (right: $M = -0.0007$, $SD = 0.0022$; left: $M = -0.0001$, $SD = 0.0021$) (right: $p = 0.001$, left: $p = 0.036$). Change in mVBM gray matter volume was not significantly different between the placebo and 1 mg groups. There were no significant effects of intracranial volume or age.

In univariate ANCOVAs including only participants 60 years of age and younger, we continued to observe a significant positive effect of E2 dose on mVBM gray matter volume change in both right ($F(2, 57) = 3.69$, $p < 0.032$) and left ($F(2,57) = 3.43$, $p < 0.039$) posterior hippocampal clusters. Post hoc Tukey tests showed a significantly greater change in mVBM gray matter volume in the 2 mg group (right: $M = 0.0012$, $SD = 0.0020$; left: $M = 0.0020$, $SD = 0.0034$) compared to the placebo (right: $M = -0.0002$, $SD = 0.0022$; left: $M = -0.0002$, $SD = 0.0026$) (right: $p = 0.010$, left: $p = 0.030$) and 1 mg (right: $M = -0.0007$, $SD = 0.0022$; left: $M = -0.0001$, $SD = 0.0021$) (right: $p = 0.020$, left: $p = 0.026$). Change in mVBM gray matter volume was not significantly different between the placebo and 1 mg groups.

4. Discussion

The main finding of the current study was that daily 2mg 17 β -estradiol administered over three months was associated with increased bilateral posterior hippocampal gray matter volume. This effect was seen primarily in women who received 2 mg daily 17- β estradiol with no significant change in hippocampal gray matter volume in women who received placebo or 1 mg daily 17- β estradiol. We did not observe any effects of E2 administration on total hippocampal gray matter volume.

These results support that, similar to previous findings in animal studies, estrogen may have trophic effects in the human hippocampus. Estrogen has been shown to increase the density of dendritic spines on CA1 pyramidal neurons especially following neuronal damage or estrogen loss (Woolley, 1998; Woolley and McEwen, 1992) while estrogen receptor antagonists block this effect (Lewis et al., 1995). The presence of estrogen appears to prime the neuron for new synapse creation through an increase in dendritic spines, however these new spines are only maintained following synapse activation (Phan et al., 2015).

The morphologic effects of estrogen have generally been seen in young animals and proposed to diminish with time since estrogen deprivation (Smith et al., 2010; Vedder et al., 2014). However, Hao and colleagues have demonstrated estrogen-induced dendritic spine increases in the prefrontal cortex of aged female non-human primates (Hao et al., 2006) which accord with beneficial estrogen effects on working memory (Rapp et al., 2003) and executive function (Voytko et al., 2009) performance. These results suggest that older animals may remain sensitive to the effects of estrogen on brain morphology and function. The current results provide evidence that similar sensitivity may be seen in older postmenopausal women, as a significant portion of the 2 mg E2 group were over the age of 60 and there was no a significant effect of age on changes in gray matter volume. In the non-human primate studies, the morphological effects of estrogen were only seen in cyclic (as opposed to continuous) administration (Young et al., 2013). In contrast, this study utilized continuous administration. Whether there are differential effects of cyclic vs. continuous estrogen administration on hippocampal structure in post-menopausal women requires further study.

Paralleling these structural changes, estrogen therapy in animal models and human-studies improves performance on cognitive tasks that are hippocampally mediated (Daniel et al., 2015; Jacobs et al., 1998; Verghese et al., 2000). Our results may help explain those findings, as we observe the effect of E2 administration on gray matter volume in the posterior hippocampus. Posterior regions of the hippocampus are associated with cognitive processes including episodic and spatial memory, whereas the anterior hippocampus has been associated with emotional and stress response processes (Bannerman et al., 2004; Fanselow and Dong, 2010). Positive effects of E2 in the posterior hippocampus may indicate increased synaptic plasticity in regions of the hippocampus important for cognitive processes that benefit from estrogen administration. This change in plasticity may provide a mechanism through which estrogen administration improves hippocampally-mediated cognitive performance in post-menopausal women.

This study is distinct from past work as it included prospective experimental manipulation of E2 administration and examined the effect of multiple clinically relevant E2 doses on hippocampal gray matter volume through both ROI-based and mVBM analysis. Interpretation of this study should be considered in context of the small sample size. Additionally the study groups were not age matched and there was a confounding effect of age and E2 dose with the 2 mg treatment group including significantly older participants than the placebo and 1 mg group. However, restricting analysis with a limited age range to eliminate the age difference between treatment groups did not substantially change our results.

The effects of estrogen in older women may depend on the duration of treatment and whether estrogen is unopposed or administered with progesterone or progestins. Previous studies which found hormone therapy effects on hippocampal volume have included long-term estrogen use and combined estrogen-progesterone treatment (Boccardi et al., 2006; Lord et al., 2008). Total hippocampal volume and voxel-based measures of gray matter volume changes may be assessable on different time scales with voxel-based methods detecting changes after short-term use and total volume changes only seen after long-term use. Alternatively, hippocampal volume may be differentially affected by estrogen and progesterone. The use of short-term unopposed E2 in this study precludes the examination of the effects of long-term use or combined treatment.

This study did not include imaging approaches that allow examination of gray matter volume in specific hippocampal subfields. As hippocampal subfields have different roles in cognitive function, the ability to examine changes by subfield may provide additional information about the functional consequences of the structural changes seen in this study. The cognitive assessment scores obtained at the screening visit for this study by design had very narrow ranges (due to inclusion and exclusion criteria requiring no evidence of mild cognitive impairment or dementia), and thus prevented meaningful analysis of the relationship between E2 effects on hippocampal structure and cognitive performance. Concurrent cognitive testing and imaging would likely contribute to the interpretation of these results and possible effects on cognitive performance.

5. Conclusions

In conclusion, postmenopausal women who were titrated to 2 mg of daily oral 17- β estradiol over 3 months exhibited increased posterior hippocampal gray matter volume. These findings support estrogen effects on hippocampal morphology that may provide a neurobiological basis for the beneficial effect of estrogen treatment on hippocampally-mediated cognitive function in postmenopausal women. Future work should examine whether the current findings of structural effects of E2 on the hippocampus are related to improvement in cognitive performance. This work may inform novel pharmacological and cognitive training approaches to maintaining cognitive function in aging women.

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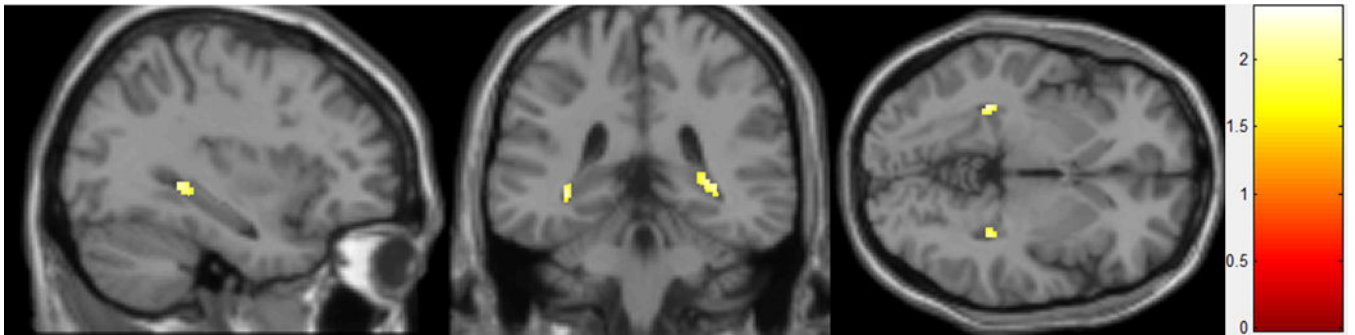


Figure 1. Estradiol Effects on Gray Matter Volume

Clusters with significant (corrected $p < 0.05$) increase in gray matter volume with increasing estradiol dose.

Table 1

Participant Age, Menopausal Symptoms, and Cognitive Assessment

	17β-Estradiol												
	placebo (n = 21)						2 mg (n = 33)						
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	One-way ANOVA (E2 Groups)
Age	56.76	2.62	52.00	60.00	55.52	2.56	51.00	59.00	61.85	6.37	53.00	74.00	F (2, 72) = 12.58, p < 0.001
MSC	19.29	10.18	6.00	49.00	15.71	9.00	4.00	37.00	14.72	9.61	1.00	41.00	F (2, 72) = 1.49, p = 0.23
DRS	141.24	2.12	137.00	144.00	141.24	1.92	137.00	144.00	139.97	8.90	92.00	144.00	F (2, 72) = 0.39, p = 0.67
GDS	1.52	0.51	1.00	2.00	1.48	0.51	1.00	2.00	1.73	0.45	1.00	2.00	F (2, 72) = 2.07, p = 0.13
BCRS	9.14	1.20	7.00	11.00	9.29	1.45	8.00	13.00	9.55	1.23	8.00	12.00	F (2, 72) = 0.68, p = 0.51
MMSE	28.90	1.41	26.00	30.00	29.10	1.04	26.00	30.00	29.27	0.98	26.00	30.00	F (2, 72) = 0.67, p = 0.51

Comparison across the three treatment groups (placebo, 1 mg, and 2 mg) for age, menopausal symptoms (MSC = Menopause Symptom Checklist total score) and cognitive assessments (DRS = Mattis Dementia Rating Scale 2; GDS = Global Deterioration Scale; BCRS = Brief Cognitive Rating Scale; MMSE = Mini-Mental State Exam). Differences in scores for the treatment groups were assessed using one-way ANOVA; the only significant ($\alpha = 0.05$) difference between groups was in age.