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Postmenopausal hormone treatment alters neural pathways but does not improve verbal cognitive function

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Abstract

Objective—Cognitive outcomes in trials of postmenopausal hormone treatment have been inconsistent. Differing outcomes may be attributed to hormone formulation, treatment duration and timing, and differential cognitive domain effects. We previously demonstrated treatment benefits on visual cognitive function. In the current study, we describe the effects of hormone treatment on verbal outcomes in the same women, seeking to understand the effects of prior versus current hormone treatment on verbal function.

Methods—This is a cross-sectional evaluation of 57 women (38 hormone users [25 prior long-term users and 13 current users] and 19 never-users). Hormone users took identical formulations of estrogen or estrogen + progestin (0.625 mg/day conjugated equine estrogens with or without medroxyprogesterone acetate) for at least 10 years, beginning within 2 years of menopause. Women were evaluated with tests of verbal function and functional MRI of a verbal discrimination task.

Results—All women scored similarly on assessments of verbal function (Hopkins Verbal Learning Test and a verbal discrimination task performed during the fMRI scanning session), however women ever-treated with hormones had more left inferior frontal ($T = 3.72$; $p < 0.001$) and right prefrontal cortex ($T = 3.53$; $p < 0.001$) activation during the verbal task. Hormone-treated women performed slightly worse on the verbal discrimination task (mean accuracy 81.72 ± 11.57 ever-treated, 85.30 ± 5.87 never-treated, $p = 0.14$), took longer to respond (mean reaction time 1.10 ± 0.17 seconds ever-treated, 1.02 ± 0.11 never-treated, $p = 0.03$), and remembered fewer previously viewed words (mean accuracy 62.21 ± 8.73 ever-treated, 65.45 ± 7.49 never-treated, $p = 0.18$). Increased posterior cingulate activity was associated with longer response times ($R = 0.323$, $p = 0.015$) and worse delayed verbal recall ($R = -0.328$, $p = 0.048$), suggesting that increased activation was associated with less efficient cognitive processing. We did not detect

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between group differences in activation in the left prefrontal cortex, superior frontal cortex, thalamus, or occipital/parietal junction.

Conclusions—Although current and past hormone treatment was associated with differences in neural pathways used during verbal discrimination, verbal function was not higher compared to never-users.

Keywords

Menopause; Hormone treatment; fMRI; verbal cognition; verbal processing

INTRODUCTION

Several randomized controlled trials of hormone therapy have yielded results indicating that there is little benefit of hormone use on postmenopausal cognition. However there is still evidence that hormone treatment may have positive effects on some cognitive domains under certain conditions defined by factors such as hormone formulation, dose, and treatment timing^{1–3}. Results from animal studies provide compelling evidence of deleterious effects of estrogen depletion on the brain, and several observational studies have upheld the hypothesis that hormone treatment may positively impact cognitive functions after menopause^{2, 4–10}. Similarly, early results from the new Early vs Late Intervention Trial with Estradiol (ELITE) study indicate that postmenopausal estradiol treatment reduces negative effects of stress on working memory in a group of women with a mean age in their mid sixties¹¹. However cognitive outcomes in randomized clinical trials have not consistently benefited from treatment. Variability of cognitive outcomes between trials has been attributed to a number of factors, including hormone dose or formulation, treatment timing and duration, study participant age, and differences in study methodologies. Importantly, methods of cognitive assessment differ greatly across studies, and may not separately assess verbal, visual, and executive cognitive domains. It is therefore not clear whether hormone treatment uniformly affects cognitive function across domains, even in those studies demonstrating positive cognitive outcomes. We previously demonstrated a positive effect of at least 10 years of estrogen (ET) or estrogen + progestin (EPT) treatment on visual cognitive function in women who began treatment soon after menopause¹². In the current study, we describe the effects of hormone treatment (ET or EPT) on verbal cognitive outcomes in the same group of women.

While many observational studies have suggested positive cognitive effects of postmenopausal hormone treatment, results from randomized trials have been variable and less encouraging. The Women's Health Initiative Memory Study (WHIMS) found that in women older than 65 years, the risk of dementia and cognitive decline was increased in those who were randomized to either estrogen alone or combined estrogen and progestin versus placebo^{13–15}, however the results of the study are not necessarily generalizable to the younger peri-menopausal population or to newly post-menopausal women. A follow-up analysis to the WHIMS study found no cognitive risk (or benefit) in women who had begun hormone treatment at age 50–54¹⁶. Cognitive function was assessed in the WHIMS study using the Modified Mini-Mental State Examination (3MSE) of global cognitive function, and did not allow for comparison of hormone effects on distinct cognitive domains. A later

cognition-specific addition to this study, the Women's Health Initiative Study of Cognitive Aging (WHISCA) did not find benefits to visual or verbal cognitive function from treatment with estrogen or combined hormone treatment in the same group of women, and saw modest decreases in some tests of verbal ability after 3 years of treatment¹⁷.

Many studies measuring effects of hormone treatment on cognitive outcomes use a global measure of executive function or risk of dementia as a cognitive endpoint, although a subset of studies separately assessed distinct cognitive domains. One study of 68 women found that women treated with 17 β -estradiol had better verbal memory performance than women treated with conjugated equine estrogens (CEE). However the Kronos Early Estrogen Prevention Study (KEEPS) recently reported results from their ancillary cognition study, and found that compared to placebo, neither CEE nor E2 with micronized progesterone had a positive or negative effect on verbal or visual cognitive function in women beginning treatment within a few years of the menopause transition and after four years of treatment^{18, 19}.

Across cognitive domains, verbally-based functions have been shown to be impacted by hormonal fluctuations associated with menopause^{20, 21}. We previously demonstrated that verbal function is more influenced by women's menopausal status than by age²². Specifically, in a cross-sectional sample of untreated pre-, peri-, and postmenopausal women, reduced verbal fluency was noted with advancing menopause status and was correlated with FSH levels and regional brain activation patterns, but was not associated with age²². Despite the evidence that verbal function is impacted by circulating hormone levels, trials of hormone treatment, even when limited to women in early menopausal years, show inconsistent verbal outcomes. A prospective observational study of perimenopausal women found that early, continuous hormone users had enhanced verbal memory performance compared to never-users, as measured with hippocampal activation on fMRI during a verbal memory test²³. However a randomized trial of hysterectomized, middle-aged, post-menopausal women found no immediate verbal cognitive benefit after 6 months of estrogen treatment²⁴. Similarly, in a trial of older post-menopausal women (ages 61–87), combined estrogen and progesterone treatment did not improve verbal recall after 2 years of treatment, although continued hormone treatment did appear to slow declines in verbal ability in subsequent years, only in women who scored above average in initial baseline assessments²⁵. While these findings are not consistent with the critical window hypothesis, they suggest that exposure to hormone therapy even when continued long after menopause may benefit women with normal verbal cognitive function.

The present study is a cross-sectional evaluation of verbal memory, using neuropsychological testing and functional magnetic resonance imaging (fMRI) of a verbal discrimination task, in early-initiated, long-term, current and past hormone users, compared to never-users. We sought to assess verbal cognitive function in post-menopausal women with prior versus current hormone use, and hypothesized that both current and prior hormone treatment would be associated with higher verbal cognition.

METHODS

This was a cross-sectional study of post-menopausal early-initiated long-term hormone users, both past and current, compared to never-users. The University of Michigan Institutional Review Board approved all procedures, and written informed consent was obtained from all participants.

Participants

Healthy, right-handed post-menopausal women over the age of 60 years were recruited by advertisement or through the University of Michigan Women's Health Registry (27, 28). Participants underwent medical, psychiatric, & physiological screening prior to study participation. Screening laboratory tests included electrolytes, glucose, complete blood count, TSH, and estradiol, and a neuropsychological battery of tests was given to exclude the presence of dementia, including the Mini-Mental State Examination²⁶, a brief screening measure of dementia, the Shipley Institute of Living Scale²⁷, a short estimate of intellectual power, and the Geriatric Depression Rating Scale²⁸, to exclude the presence of depression. Women were excluded for acute illness, diabetes, heart disease, cancer, or stroke, neurologic, psychiatric, or acute medical illness, centrally acting medications, intermittent estrogen or phytoestrogen supplements, tobacco use within five years, and contraindications to MRI (claustrophobia, pacemakers, or metallic implants or devices), however women with hypertension or hypercholesterolemia were included. Women had either taken hormones continuously for a minimum of ten years or had never received hormone therapy. All hormone users had begun therapy within two years of menopause, which was defined as the absence of menses for one year, the onset of severe menopausal symptoms following hysterectomy, or the surgical removal of ovaries at the time of hysterectomy. All hormone users received 0.625 mg/day conjugated equine estrogens (Premarin, Wyeth Pharmaceuticals, Philadelphia, PA) with (EPT users) or without (ET users) cyclic or continuous medroxyprogesterone acetate (Provera, Pfizer, New York, NY, or Prempro, Wyeth Pharmaceuticals). Fifty-seven women were included in the study: 17 ET users, 21 EPT users, and 19 never-users. Women in the hormone therapy group (n=38) included current (13) and prior (25) long-term users. For women no longer taking hormones, the mean time since treatment ended was 1.88 ± 1.67 years. Estradiol concentrations were measured with a modified off-line ACS:180 E₂-6 immunoassay (Bayer Diagnostics Corp., Northwood, MA).

fMRI Verbal Discrimination & Recall Task

Episodic verbal discrimination processes were assessed during the fMRI scanning session using the levels of processing verbal discrimination paradigm, in which the women were asked to judge words based on physical characteristics (shallow processing) or on word meanings (deep processing) (44). Sixteen lists of twelve words each were equated for letter length and frequency, with four counterbalanced lists presented in each of four scanning runs. Each list was preceded by eight seconds of instructions, and each word was presented for 1.5 sec with a 1.5-second interstimulus interval. In the phonemic condition, participants decided whether each word was presented in uppercase or lowercase letters (shallow processing). In the semantic condition, participants decided whether each word represented

an abstract or concrete concept (deep processing). Each list contained equal numbers of uppercase, lowercase, abstract, and concrete words. Responses were made by button-press with the right hand. Verbal tasks were presented on a computer monitor through radio frequency-shielded goggles mounted to a head coil (Resonance Technology Inc., Northridge, CA). To minimize performance differences, participants practiced tasks before the scanning session until they achieved at least 70% accuracy. After the scanning session participants performed a verbal recall task, in which they were presented with a series of words, half of which had been previously presented during the verbal discrimination task, and asked to indicate if they recalled seeing each word during the verbal discrimination task.

Neuropsychological Assessment of Verbal Cognitive Function

Verbal cognitive function was assessed using the Hopkins Verbal Learning Test (HVLT-R²⁹), administered by a trained psychometrician, which assess verbal list learning, recall, and recognition. In this task, a list of 12 words is presented three times with participants asked to recall the words after each presentation, with the total number of words recalled across all three trials recorded. Participants are asked to recall the words again after 20 minutes, and asked to indicate recognized words from a list consisting of presented and unrepresented words.

fMRI Acquisition and Processing

Scans were acquired using a 3-T whole-body MRI scanner (General Electric, Milwaukee, WI) equipped with a standard head coil. Anatomical MRI scans were acquired axially with a spoiled gradient recalled three-dimensional volumetric acquisition (repetition time, 9.6 ms; echo time, 3.3 ms; inversion recovery preparation, 200 ms; flip angle, 17°; bandwidth, 15.63 hertz; 24-cm field of view; 1.5-mm slice thickness; 106–110 slices; 256 × 256 matrix; two excitations). fMRI acquisition was sensitized for the blood oxygen level-dependent (BOLD) effect using a T2*-weighted single-shot spiral pulse sequence with 32 oblique-axial slices prescribed to be approximately parallel to the anterior commissure-posterior commissure line (spiral gradient echo: echo time, 25 ms; repetition time, 2,000 ms; flip angle, 60°; 4-mm-thick contiguous slices; 24-cm field of view; 64 × 64 image matrix). Image reconstruction included processing steps to remove distortions caused by magnetic field inhomogeneity and other sources of misalignment to the structural data³⁰. Data were sinc-interpolated in time, slice by slice, to correct for the staggered sequence of slice acquisition³¹. The first four functional volumes of each run were discarded to remove magnetic saturation effects, and the remaining images were realigned to the fifth volume to eliminate movement artifacts using SPM2-based algorithms³². Realignment parameters for each participant were examined to ensure that head movement did not exceed 2 mm. Anatomical and functional images were coregistered to each other through rigid body affine transformation using a mutual information algorithm, as previously described³³. The participant's MRI was spatially normalized into standard stereotactic space via linear and nonlinear warping, to a minimum deformation template derived from T1-weighted magnetic resonance images from 25 normal older women, created by the Imaging of Dementia and Aging Laboratory at the University of California, Davis³⁴. The transformation matrix was applied to all functional images, and a three-dimensional Gaussian smoothing kernel set at 8

mm full-width at half-maximum was applied to accommodate for residual anatomical variability and improve signal-to-noise ratios.

Data Analysis

fMRI data analyses were conducted using the general linear model (GLM) in statistical parametric mapping imaging software (SPM, Department of Cognitive Neurology, Wellcome, London, UK). For the first-level analysis, contrast images were generated for each participant to assess activation differences between task conditions. For verbal processing task image analysis, initial contrast images (deep encoding-shallow encoding) were subtracted to isolate the deep encoding component, which was then used to assess effects of hormone treatment on deep verbal discrimination and encoding. We performed initial 1 sample t-tests to evaluate effects of hormone treatment status in our study sample, including regions as significant with a false discovery rate (FDR)-corrected $p < 0.000$, using cluster-level threshold of $p = 0.001$. 2 sample t-tests were then performed to compare activation patterns between hormone ever-users and never-users, and between past and current hormone users, including regions as significant if they were present in the initial 1 sample t-test analysis and $p < 0.000$ before correction for multiple comparisons. We only examined regions significantly different at the voxel level to reduce concerns of false positives associated with cluster-level comparisons³⁵. Beta values were extracted from regions meeting these criteria for significant activation for use in secondary analyses. Clinical, demographic, and behavioral variables were compared between hormone treatment groups using 2 sample T-test analyses. Associations between clinical, behavioral, and regional activation were determined using Pearson correlational analyses. Because mean age was nearly identical across all groups, and women who were ever or never treated with hormones had similar levels of education, we did not include these variables as covariates in analyses.

RESULTS

Demographic & clinical characteristics

Demographic and clinical characteristics are provided in Table 1. Mean age for the entire group ($n = 57$) was 66.25 ± 5.50 (SD) years, and women had on average 16.39 ± 2.83 years of education. The mean time since menopause was 17.94 ± 6.31 years. Age was similar between never- and ever- treated women (66.22 ± 5.08 and 66.26 ± 5.74 years, $p = 0.979$), and between current and past treatment groups (65.69 ± 6.25 and 66.56 ± 5.57 years, $p = 0.665$). Education did not differ between never and ever treated women (16.63 ± 2.95 and 16.26 ± 2.80 years, $p = 0.647$), but currently treated women had fewer years of education than past treated women (14.92 ± 2.84 and 16.96 ± 2.56 years, $p = 0.031$). Years since menopause was similar between never and ever treated women (16.47 ± 5.82 and 18.62 ± 6.49 years, $p = 0.249$), and between currently and past treated women (19.54 ± 8.03 and 18.13 ± 5.62 years, $p = 0.579$). Mean treatment duration for all women ever treated with hormones was 16.39 ± 6.21 years, and did not differ significantly between current and past treated women (17.23 ± 7.11 and 15.96 ± 5.81 years, $p = 0.125$). Estradiol levels were different between never and ever treated women (16.54 ± 6.42 and 24.37 pg/mL, $p = 0.030$),

and between current and past treatment groups (35.92 ± 23.90 and 18.36 ± 14.03 pg/mL, $p = 0.026$).

Neural activation during verbal discrimination task

Across the entire group, semantic discrimination during the fMRI verbal processing task elicited neural activation in cognitive processing regions of the brain (Table 2A). These regions included the left inferior frontal cortex (Montreal Neurological Institute (MNI) coordinates $-44, 22, -4$, $T = 7.31$, FDR-corrected $p < 0.001$), bilateral prefrontal cortex ($50, 28, 20$, $T = 6.32$, $p < 0.001$ right; $-40, 20, 20$, $T = 8.14$, $p < 0.001$ left), superior frontal cortex ($-2, 30, 50$, $T = 6.92$, $p < 0.001$), thalamus ($-12, -6, 16$, $T = 5.15$, $p < 0.001$), and the left occipital/parietal junction ($-26, -62, 32$, $T = 5.12$, $p < 0.001$). When compared to never-treated women, women ever treated with hormones had more activation during the task in the left inferior frontal cortex ($-54, 14, -6$, $T = 3.72$, uncorrected $p < 0.001$), and right prefrontal cortex ($6, 54, -2$, $T = 3.53$, uncorrected $p < 0.001$), while never-treated women had no regions with significantly more activation during the task than ever-treated women (Table 2B). Past and current hormone users did not have significant differences in activation patterns (Table 2C).

Verbal cognitive function & behavioral correlates

All groups had similar accuracy on the fMRI deep verbal discrimination and verbal recall tasks (verbal discrimination: $85.30 \pm 5.87\%$ correct never-treated, $81.72 \pm 11.57\%$ ever-treated, $p = 0.137$; $83.82 \pm 10.05\%$ currently treated, $80.79 \pm 12.25\%$ past treated, $p = 0.478$; verbal recall: $65.45 \pm 7.49\%$ never-treated, $62.21 \pm 8.73\%$ ever-treated, $p = 0.181$; $61.56 \pm 10.25\%$ current treatment, $62.55 \pm 8.05\%$ past treatment, $p = 0.746$). Ever-treated women took longer than never-treated women to form responses to both the verbal discrimination and verbal recall tasks (verbal discrimination reaction time: 1.02 ± 0.11 s never-treated, 1.10 ± 0.17 s ever-treated, $p = 0.035$; verbal recall reaction time: 1.03 ± 0.13 s never-treated, 1.16 ± 0.16 s ever-treated, $p = 0.007$), while current and past treated women had similar reaction times for both tasks (verbal discrimination reaction time: 1.03 ± 0.16 s current treatment, 1.14 ± 0.16 s past treatment, $p = 0.68$; verbal memory reaction time: 1.17 ± 0.15 s current treatment, 1.15 ± 0.17 s past treatment, $p = 0.724$) (Table 3).

While there were no differences between groups on the neuropsychological measures of cognitive function (Table 3), HVLIT delayed verbal memory scores were negatively correlated with activation during the verbal discrimination task in the posterior cingulate ($R = -0.328$, $p = 0.048$). Posterior cingulate activation during the task was also correlated with reaction times on the verbal discrimination task ($R = 0.323$, $p = 0.015$) and verbal memory task ($R = 0.272$, $p = 0.043$). Estradiol levels were negatively correlated with the HVLIT verbal learning test ($R = -0.270$, $p = 0.044$) (Figure 1).

Discussion

Deficiencies in cognitive functioning experienced during and after menopause are associated with changes in the hormone environment and reduced circulating estrogen levels, independent of age²². However trials of menopausal hormone treatment have not

consistently demonstrated a positive impact on cognitive performance. Timing of treatment onset, treatment duration, and hormone formulation have been implicated in differences in cognitive outcomes between studies. In the current study, we addressed verbal cognitive function in women who had previous or current use of hormone treatment for at least ten years, and beginning within two years of menopause, compared to women who had never experienced postmenopausal hormone treatment. We used a combination of neuropsychological assessments of verbal function and regional synaptic activity during a test of verbal discrimination as indirectly assessed with functional MR blood-oxygen-level dependent (BOLD) imaging.

Women in the study performed similarly in neuropsychological assessments of verbal function, however women currently or previously treated with hormones (E only or E+P) had more regional brain activity during the verbal discrimination task than women never treated with hormones. Increased regional activity was localized to the left inferior frontal cortex and right prefrontal cortex, which are areas associated with verbal cognitive processing and executive control of cognitive function. Functionally, women ever treated with hormones performed slightly worse on the deep verbal processing task, took longer to formulate responses, and were less accurate at remembering previously viewed words than women never treated with hormones. Additionally, increased regional brain activity in the posterior cingulate during the verbal processing task was positively correlated with longer response times, and was associated with worse delayed verbal recall task performance, suggesting that increased activation was associated with less efficient cognitive processing. This pattern is consistent with previous findings from our group that progression through the menopause transition is associated with increased activation during the same verbal processing task and with worse performance on measures of verbal cognitive function, again consistent with less efficient neural processing²².

Overall, current and past hormone users performed similarly on tests of verbal function and on the verbal discrimination task during the scanning session, and had similar activation patterns during the verbal discrimination task. The “critical window hypothesis” suggests that treatment with hormones near the time of menopause may have lasting cognitive benefits that persists beyond treatment discontinuation^{2, 36}. This hypothesis is supported by an animal model demonstrating that short-term exposure to estradiol in middle-aged rats improved memory up to 7 months after exposure³⁷, and by a cross-sectional analysis of women aged 60–89 that found significantly better verbal memory in women with any history of prior hormone therapy³⁸. However not all trials have supported this hypothesis, for example in a study of women with 7 years of early menopausal CEE with or without MPA treatment, there were no cognitive differences compared to placebo 7 years after ending treatment³⁹. We did not find behavioral differences in verbal cognitive function between current and past hormone users in our study. However, differences in regional activity in cognitive association networks (prefrontal, thalamic, and occipital areas) during the verbal discrimination task suggest that there may be underlying neurobiological differences between current and past hormone user groups, albeit not linearly associated with neuropsychological measures of verbal function and with implications not clarified in the present study.

In our sample, hormone formulation may be a contributing factor to relatively worse performance in women ever treated compared to those never treated with hormones. A previous study comparing CEE to 17 β -estradiol found more favorable effects of 17 β -estradiol on verbal memory performance in women at risk for AD, and conversely, a pattern of increased neural activation during a visual encoding task in both CEE and estradiol users compared to non-users, interpreted as better neural function in the hormone-using groups^{18, 40, 41}. This is consistent with animal studies that have demonstrated that E2 improves memory by promoting synaptic density and increasing long-term potentiation in the hippocampus of ovariectomized rats^{42, 43}. A related study in postmenopausal women has determined that depleting E2 with an aromatase inhibitor decreased hippocampal activation during memory encoding and comparatively worse scores for verbal over visual recollection⁴⁴. However the recently released cognitive results from the Kronos early estrogen prevention study (KEEPS) study found that neither CEE nor E2, along with progesterone improved verbal cognitive function compared to placebo, after 4 years of treatment initiated within 3 years of final menstrual period¹⁹.

We previously found that ever-use of postmenopausal hormones was associated with increased regional brain activation during a visual memory task, and that regional activation was associated with better performance on the task, in the same group of women described in the current study¹². Also in this same group of women, we found increased resting cholinergic activity in the hippocampus and the posterior cingulate in E+P treated women, consistent with preservation of cholinergic activity and neuronal integrity in the E+P group⁴⁵. While the verbal discrimination task used in this study was also associated with greater regional activation in ever-treated women, behavioral data indicates that this increased activation was associated with worse performance on the verbal task. Ever-treated women also had longer response times than never-treated women, and the corresponding activity in the brain regions used for cognitive processing may indicate increased effort required to formulate responses to words presented during the task. Differential effects of hormone use across cognitive domains has been reported in other studies. A study of cognitive function in women who had experienced premature menopause found that hormone treatment at the time of menopause was associated with better visual cognitive function but worse verbal function after age 65⁴⁶. A separate study of postmenopausal women with cognitive complaints found no effect of CEE with or without MPA treatment on visual function but modest negative effects on verbal cognition, compared to placebo⁴⁷. Progestin may also have contributed to our findings of relatively worse verbal function in hormone ever-users. A study of cognitive function after 12 weeks of treatment with CEE plus MPA, micronized progesterone, or placebo found that women treated with CEE + micronized progesterone outscored other treatment groups in tests of working memory, but demonstrated significantly decreased delayed verbal memory compared to pre-treatment testing⁴⁸.

Strengths of this study include the comparison of consistent hormone users to never-users, with separate analyses of women with current or past hormone use. Our study included both ET (for women with hysterectomy) and EPT. All women were on identical hormone doses and formulations. This assessment of long-term use required a cross-sectional design because randomization to long-term hormone use was not feasible. Our study examined the

effects of chronic, rather than acute, hormone use and thus allowed inclusion of both current and past hormone users; women falling into these groups were demographically similar. We did not include a comparison group of women who began hormone use well past menopause, but limiting our study to women who began hormone use early allowed us to include only women who used hormones long-term. A limitation of this design is the inability to separate the effects of early initiation from those of long-term use of hormones, and the small sample size, however sensitivity of fMRI imaging technologies allows detection of small differences in regional brain activation between groups⁴⁹. Onset of menopause was self-defined, although whenever possible, we confirmed dates with healthcare records.

Conclusions

Our study results indicate that postmenopausal hormone use may not benefit verbal cognitive function, although current and past hormone use is associated with differences in neural pathways used while assessing verbal semantic distinctions. In the same group of women we previously found evidence of improved visual cognitive function in both ET and EPT users, suggesting that the cognitive impacts of postmenopausal hormone use are domain specific.

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References

1. Espeland MA, Rapp SR, Manson JE, et al. Long-term Effects on Cognitive Trajectories of Postmenopausal Hormone Therapy in Two Age Groups. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2017; 72(6):838–45.
2. McCarrey AC, Resnick SM. Postmenopausal hormone therapy and cognition. *Hormones and behavior*. 2015; 74:167–72. [PubMed: 25935728]
3. Fischer B, Gleason C, Asthana S. Effects of hormone therapy on cognition and mood. *Fertility and sterility*. 2014; 101(4):898–904. [PubMed: 24680649]
4. Singh M, Meyer EM, Millard WJ, Simpkins JW. Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. *Brain research*. 1994; 644(2):305–12. [PubMed: 8050041]
5. Roberts JA, Gilardi KV, Lasley B, Rapp PR. Reproductive senescence predicts cognitive decline in aged female monkeys. *Neuroreport*. 1997; 8(8):2047–51. [PubMed: 9223100]
6. Lokkegaard E, Pedersen AT, Laursen P, Loft IP, Larsen S, Jorgensen T. The influence of hormone replacement therapy on the aging-related change in cognitive performance. Analysis based on a Danish cohort study. *Maturitas*. 2002; 42(3):209–18. [PubMed: 12161045]
7. Low LF, Anstey KJ. Hormone replacement therapy and cognitive performance in postmenopausal women--a review by cognitive domain. *Neuroscience and biobehavioral reviews*. 2006; 30(1):66–84. [PubMed: 16122800]

8. Haskell SG, Richardson ED, Horwitz RI. The effect of estrogen replacement therapy on cognitive function in women: a critical review of the literature. *Journal of clinical epidemiology*. 1997; 50(11):1249–64. [PubMed: 9393381]
9. Yonker JE, Adolfsson R, Eriksson E, Hellstrand M, Nilsson LG, Herlitz A. Verified hormone therapy improves episodic memory performance in healthy postmenopausal women. *Neuropsychology, development, and cognition Section B, Aging, neuropsychology and cognition*. 2006; 13(3–4):291–307.
10. Carlson MC, Zandi PP, Plassman BL, et al. Hormone replacement therapy and reduced cognitive decline in older women: the Cache County Study. *Neurology*. 2001; 57(12):2210–6. [PubMed: 11756599]
11. Herrera AY, Hodis HN, Mack WJ, Mather M. Estradiol Therapy After Menopause Mitigates Effects of Stress on Cortisol and Working Memory. *The Journal of clinical endocrinology and metabolism*. 2017; 102(12):4457–66. [PubMed: 29106594]
12. Berent-Spillson A, Persad CC, Love T, et al. Early menopausal hormone use influences brain regions used for visual working memory. *Menopause*. 2010; 17(4):692–9. [PubMed: 20300040]
13. Craig MC, Maki PM, Murphy DG. The Women's Health Initiative Memory Study: findings and implications for treatment. *The Lancet Neurology*. 2005; 4(3):190–4. [PubMed: 15721829]
14. Shumaker SA, Legault C, Kuller L, et al. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. *Jama*. 2004; 291(24):2947–58. [PubMed: 15213206]
15. Coker LH, Espeland MA, Rapp SR, et al. Postmenopausal hormone therapy and cognitive outcomes: the Women's Health Initiative Memory Study (WHIMS). *The Journal of steroid biochemistry and molecular biology*. 2010; 118(4–5):304–10. [PubMed: 19932751]
16. Espeland MA, Rapp SR, Manson JE, et al. Long-term Effects on Cognitive Trajectories of Postmenopausal Hormone Therapy in Two Age Groups. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2016
17. Resnick SM, Maki PM, Rapp SR, et al. Effects of combination estrogen plus progestin hormone treatment on cognition and affect. *The Journal of clinical endocrinology and metabolism*. 2006; 91(5):1802–10. [PubMed: 16522699]
18. Wroolie TE, Kenna HA, Williams KE, et al. Differences in verbal memory performance in postmenopausal women receiving hormone therapy: 17beta-estradiol versus conjugated equine estrogens. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry*. 2011; 19(9):792–802. [PubMed: 21873835]
19. Gleason CE, Dowling NM, Wharton W, et al. Effects of Hormone Therapy on Cognition and Mood in Recently Postmenopausal Women: Findings from the Randomized, Controlled KEEPS-Cognitive and Affective Study. *PLoS medicine*. 2015; 12(6):e1001833. discussion e. [PubMed: 26035291]
20. Maki PM. Verbal memory and menopause. *Maturitas*. 2015; 82(3):288–90. [PubMed: 26433715]
21. Weber MT, Maki PM, McDermott MP. Cognition and mood in perimenopause: A systematic review and meta-analysis. *The Journal of steroid biochemistry and molecular biology*. 2013
22. Berent-Spillson A, Persad CC, Love T, et al. Hormonal Environment Affects Cognition Independent of Age during the Menopause Transition. *The Journal of clinical endocrinology and metabolism*. 2012
23. Maki PM, Dennerstein L, Clark M, et al. Perimenopausal use of hormone therapy is associated with enhanced memory and hippocampal function later in life. *Brain research*. 2011; 1379:232–43. [PubMed: 21078303]
24. Gorenstein C, Renno J Jr, Vieira Filho AH, et al. Estrogen replacement therapy and cognitive functions in healthy postmenopausal women: a randomized trial. *Archives of women's mental health*. 2011; 14(5):367–73.
25. Tierney MC, Oh P, Moineddin R, et al. A randomized double-blind trial of the effects of hormone therapy on delayed verbal recall in older women. *Psychoneuroendocrinology*. 2009; 34(7):1065–74. [PubMed: 19297102]

26. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research*. 1975; 12(3):189–98. [PubMed: 1202204]
27. Shipley WC. *Institute of Living Scale*. Los Angeles: Western Psychological Services; 1946.
28. Yesavage JA, Rose TL, D L. Validity of the geriatric depression scale in subjects with senile dementia. Palo Alto: Clinical Diagnostic and Rehabilitation Unit, Veterans Administrative Medical Center; 1981.
29. Brandt J. The Hopkins verbal learning test: Development of a new memory test with six equivalent forms. *Clinical Neuropsychologist*. 1991; 5(2):125–42.
30. Noll D, Stenger V, Vasquez A, Peltier S. Spiral scanning in functional MRI. Moonen C, Bandettini P, editors Heidelberg: Springer-Verlag; 1999.
31. Acquire C, D'Esposito M. Experimental design for brain fMRI. Moonen C, Bandettini P, editors Heidelberg: Springer-Verlag; 1999.
32. Friston K, Ashburner J, Frith C, Poline J-B, Heather J, Frackowiak R. Spatial registration and normalization of images. *Human brain mapping*. 1995; 2:165–89.
33. Meyer CR, Boes JL, Kim B, et al. Demonstration of accuracy and clinical versatility of mutual information for automatic multimodality image fusion using affine and thin-plate spline warped geometric deformations. *Med Image Anal*. 1997; 1(3):195–206. [PubMed: 9873906]
34. Sun FT, Schriber RA, Greenia JM, He J, Gitcho A, Jagust WJ. Automated template-based PET region of interest analyses in the aging brain. *Neuroimage*. 2007; 34(2):608–17. [PubMed: 17112749]
35. Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proceedings of the National Academy of Sciences of the United States of America*. 2016; 113(28):7900–5. [PubMed: 27357684]
36. Daniel JM, Witty CF, Rodgers SP. Long-term consequences of estrogens administered in midlife on female cognitive aging. *Hormones and behavior*. 2015; 74:77–85. [PubMed: 25917862]
37. Rodgers SP, Bohacek J, Daniel JM. Transient estradiol exposure during middle age in ovariectomized rats exerts lasting effects on cognitive function and the hippocampus. *Endocrinology*. 2010; 151(3):1194–203. [PubMed: 20068005]
38. Tierney MC, Ryan J, Ancelin ML, et al. Lifelong estrogen exposure and memory in older postmenopausal women. *Journal of Alzheimer's disease : JAD*. 2013; 34(3):601–8.
39. Espeland MA, Shumaker SA, Leng I, et al. Long-term effects on cognitive function of postmenopausal hormone therapy prescribed to women aged 50 to 55 years. *JAMA internal medicine*. 2013; 173(15):1429–36. [PubMed: 23797469]
40. Silverman DH, Geist CL, Kenna HA, et al. Differences in regional brain metabolism associated with specific formulations of hormone therapy in postmenopausal women at risk for AD. *Psychoneuroendocrinology*. 2011; 36(4):502–13. [PubMed: 20810219]
41. Gleason CE, Schmitz TW, Hess T, et al. Hormone effects on fMRI and cognitive measures of encoding: importance of hormone preparation. *Neurology*. 2006; 67(11):2039–41. [PubMed: 17159116]
42. Li C, Brake WG, Romeo RD, et al. Estrogen alters hippocampal dendritic spine shape and enhances synaptic protein immunoreactivity and spatial memory in female mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(7):2185–90. [PubMed: 14766964]
43. Smith CC, Vedder LC, McMahon LL. Estradiol and the relationship between dendritic spines, NR2B containing NMDA receptors, and the magnitude of long-term potentiation at hippocampal CA3-CA1 synapses. *Psychoneuroendocrinology*. 2009; 34(Suppl 1):S130–42. [PubMed: 19596521]
44. Bayer J, Rune G, Schultz H, et al. The effect of estrogen synthesis inhibition on hippocampal memory. *Psychoneuroendocrinology*. 2015; 56:213–25. [PubMed: 25863445]
45. Smith YR, Bowen L, Love TM, et al. Early initiation of hormone therapy in menopausal women is associated with increased hippocampal and posterior cingulate cholinergic activity. *The Journal of clinical endocrinology and metabolism*. 2011; 96(11):E1761–70. [PubMed: 21865354]

46. Ryan J, Scali J, Carriere I, et al. Impact of a premature menopause on cognitive function in later life. *BJOG : an international journal of obstetrics and gynaecology*. 2014; 121(13):1729–39. [PubMed: 24802975]
47. Maki PM, Gast MJ, Vieweg AJ, Burriss SW, Yaffe K. Hormone therapy in menopausal women with cognitive complaints: a randomized, double-blind trial. *Neurology*. 2007; 69(13):1322–30. [PubMed: 17893293]
48. Sherwin BB, Grigorova M. Differential effects of estrogen and micronized progesterone or medroxyprogesterone acetate on cognition in postmenopausal women. *Fertility and sterility*. 2011; 96(2):399–403. [PubMed: 21703613]
49. Woodard JL, Seidenberg M, Nielson KA, et al. Prediction of cognitive decline in healthy older adults using fMRI. *Journal of Alzheimer's disease : JAD*. 2010; 21(3):871–85.

	L Inf Frontal Cortex		R Prefrontal Cortex		Posterior Cingulate		Estradiol	
<i>Demographics</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Age	-.185	(.177)	.023	(.867)	-.081	(.551)	.398**	(.002)
Education	-.026	(.852)	-.140	(.309)	-.102	(.450)	-.269*	(.043)
Years since menopause	-.320*	(.020)	-.067	(.637)	-.070	(.613)	.345*	(.011)
Treatment duration	-.186	(.270)	-.090	(.597)	-.002	(.992)	.450**	(.005)
Years since treatment	-.208	(.217)	-.009	(.956)	.156	(.350)	-.166	(.429)
Estradiol	.109	(.426)	.127	(.356)	.065	(.632)		
<i>fMRI tasks</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Verbal discrimination accuracy	.007	(.961)	.188	(.177)	.092	(.510)	-.242	(.078)
Verbal discrimination RT	.074	(.592)	-.109	(.430)	.323*	(.015)	.068	(.616)
Verbal memory accuracy	-.116	(.398)	.258	(.057)	.044	(.746)	-.118	(.386)
Verbal memory RT	.017	(.900)	-.073	(.595)	.272*	(.043)	.149	(.274)
<i>Neuropsychological tests</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Logical memory recall	.022	(.871)	.095	(.494)	.014	(.916)	-.150	(.271)
Logical memory delay recall	.099	(.472)	.130	(.347)	-.077	(.571)	-.253	(.059)
HVLT verbal learning	-.059	(.670)	.040	(.773)	.015	(.914)	-.270*	(.044)
HVLT delay recall	.203	(.235)	.228	(.181)	-.328*	(.048)	-.015	(.930)

Figure 1.

In the entire group of women, regional brain activation during the deep encoding component of the verbal discrimination task (top images from left to right, left inferior frontal cortex, right prefrontal cortex, and posterior cingulate, with statistical intensities as represented by the T score color bar) were correlated with demographic, verbal discrimination task, and neuropsychological tests of verbal learning & memory.

Participant characteristics

Table 1

	Entire Sample <i>n</i> = 57	Never Treated <i>n</i> = 19	Ever Treated <i>n</i> = 38	Current Treatment <i>n</i> = 13	Past Treatment <i>n</i> = 25	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	<i>p</i> **
Age	66.25 ± 5.50	66.22 ± 5.08	66.26 ± 5.74	65.69 ± 6.25	66.56 ± 5.57	0.66
Education	16.39 ± 2.83	16.63 ± 2.95	16.26 ± 2.80	14.92 ± 2.84	16.96 ± 2.56	0.03
Years Since Menopause	17.94 ± 6.31	16.47 ± 5.82	18.62 ± 6.49	19.54 ± 8.03	18.13 ± 5.62	0.58
Treatment Duration			16.39 ± 6.21	17.23 ± 7.11	15.96 ± 5.81	0.12
Estradiol (pg/mL)		16.53 ± 6.42	24.37 ± 19.60	35.92 ± 23.90	18.36 ± 14.03	0.03

* , ** : *p*-values calculated as 2-tailed significance level of independent samples *t* tests between never and ever treated women (*) or current and past treated women (**), after assessing for equal variances between groups.

Table 2

Regional brain activation patterns during verbal discrimination task

A. Effect of verbal discrimination task					
Region	Coordinates [*]	T	P _{uncorr} ^{***}	P _{FDR} ^{***}	Size (mm ³)
L inferior frontal cortex	-44, 22, -4	7.31	< 0.001	< 0.001	848
R prefrontal cortex	50, 28, 20	6.32	< 0.001	< 0.001	27312
L prefrontal cortex	-40, 20, 20	8.14	< 0.001	< 0.001	31784
Superior frontal cortex	-2, 30, 40	6.92	< 0.001	< 0.001	14792
L thalamus	-12, -6, 16	5.15	< 0.001	< 0.001	800
L occipital/parietal junction	-26, -62, 32	5.12	< 0.001	< 0.001	1328
B. Ever-treated Vs never-treated					
Region	Coordinates	T	P _{uncorr}	P _{FDR}	Size (mm ³)
<u>Ever Treated - Never Treated</u>					
L inferior frontal cortex	-54, 14, 6	3.72	< 0.001	0.673	1680
R prefrontal cortex	6, 54, -2	3.53	< 0.001	0.673	744
<u>Never Treated - Ever Treated</u>					
<i>No regions</i>					
C. Current vs past treated					
Region	Coordinates	T	P _{uncorr}	P _{FDR}	Size (mm ³)
<i>No regions</i>					

Regions activated during the verbal discrimination task deep encoding component in all women (A), and T test comparison between ever-treated and never-treated women (B) or current and past treated women (C).

* Brain region locations provided as Montreal Neurological Institute (MNI) standardized x, y, z coordinates

***, ** P values provided as uncorrected (uncorr; **) for multiple comparisons and after applying False Detection Rate (FDR; ***) correction for multiple comparisons.

Table 3

Comparison of verbal cognitive function between treatment groups

	Never Treated <i>n</i> = 19		Ever Treated <i>n</i> = 38		Current Treatment <i>n</i> = 13		Past Treatment <i>n</i> = 25	
<i>fMRI Tasks</i>	Mean ± SD		Mean ± SD	<i>p</i> [*]	Mean ± SD		Mean ± SD	<i>p</i> ^{**}
Verbal discrimination accuracy	85.30 ± 5.87		81.72 ± 11.57	0.14	83.82 ± 10.05		80.79 ± 12.25	0.48
Verbal discrimination RT	1.02 ± 0.11		1.10 ± 0.17	0.03	1.03 ± 0.16		1.14 ± 0.16	0.07
Verbal memory accuracy	65.45 ± 7.49		62.21 ± 8.73	0.18	61.56 ± 10.25		62.55 ± 8.05	0.75
Verbal memory RT	1.03 ± 0.13		1.16 ± 0.16	0.01	1.17 ± 0.15		1.15 ± 0.17	0.72
<i>Neuropsychological Tests</i>								
Logical memory recall	26.58 ± 6.64		26.95 ± 6.06	0.84	25.92 ± 7.12		27.44 ± 5.58	0.48
Logical memory delay recall	27.89 ± 6.34		26.89 ± 7.57	0.62	24.92 ± 8.47		27.84 ± 7.08	0.28
HVLT verbal learning	28.37 ± 4.57		26.95 ± 4.01	0.24	26.33 ± 3.47		27.24 ± 4.27	0.53
HVLT delay recall	10.67 ± 1.61		9.60 ± 1.83	0.09	9.29 ± 1.80		9.72 ± 1.87	0.60

^{*}, ^{**}, ^{***} p-values calculated as 2-tailed significance level of independent samples t tests between never and ever treated women (^{*}) or current and past treated women (^{**}), after assessing for equal variances between groups.