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RESEARCH ARTICLE

Estrogen, brain structure, and cognition in postmenopausal women

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Abstract

Declining estrogen levels before, during, and after menopause can affect memory and risk for Alzheimer's disease. Undesirable side effects of hormone variations emphasize a role for hormone therapy (HT) where possible benefits include a delay in the onset of dementia—yet findings are inconsistent. Effects of HT may be mediated by estrogen receptors found throughout the brain. Effects may also depend on lifestyle factors, timing of use, and genetic risk. We studied the impact of self-reported HT use on brain volume in 562 elderly women (71–94 years) with mixed cognitive status while adjusting for aforementioned factors. Covariate-adjusted voxelwise linear regression analyses using a model with 16 predictors showed HT use as positively associated with regional brain volumes, regardless of cognitive status. Examinations of other factors related to menopause, oophorectomy and hysterectomy status independently yielded positive effects on brain volume when added to our model. One interaction term, HTxBMI, out of several examined, revealed significant negative association with overall brain volume, suggesting a greater reduction in brain volume than BMI alone. Our main findings relating HT to regional brain volume were as

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hypothesized, but some exploratory analyses were not in line with existing hypotheses. Studies suggest lower levels of estrogen resulting from oophorectomy and hysterectomy affect brain volume negatively, and the addition of HT modifies the relation between BMI and brain volume positively. Effects of HT may depend on the age range assessed, motivating studies with a wider age range as well as a randomized design.

KEYWORDS
Alzheimer's disease, Brain volume, hormone therapy

1 | INTRODUCTION

By 2030, the world population of menopausal and postmenopausal women is projected to increase to 1.2 billion, with 47 million new entrants each year (Hill, 1996). Using age 50 as a proxy for menopause, about 25 million women pass through menopause each year (Hill, 1996). Perimenopause, menopause, and postmenopause all represent periods of life where many women have been considered candidates for conjugated equine estrogens (CEE) or other forms of exogenous hormone therapy (HT) to treat menopausal symptoms. HT was initially regarded as potentially protective against heart disease, osteoporosis, and dementia (Green & Simpkins, 2000; Mendelsohn, 2002) in postmenopausal women. Prescriptions for CEE fell abruptly (Kim et al., 2005) after negative reports from large multicenter trials showed equivocal effects or even increased risk of adverse health outcomes (Grady et al., 2002; Shumaker et al., 2003; Shumaker, Legault, Kuller, et al., 2004). More recently, however, studies began to re-evaluate the possible benefits of HT including stress reduction, enhancement of cardiovascular health, improvement in cognitive performance, and a delay in the onset of dementia (Herrera, Hodis, Mack, & Mather, 2017; Merlo, Spampinato, & Sortino, 2017; Speth, D'Ambra, Ji, & Sandberg, 2018).

Alzheimer's disease (AD) is the most common neurodegenerative cause of dementia; female sex is a key risk factor for AD, particularly after menopause and precipitous declines in estrogen levels (Mosconi et al., 2018; Riedel, Thompson, & Brinton, 2016). Estradiol is the most bioactive estrogen before menopause (Fischer, Gleason, & Asthana, 2014), acting on alpha and beta-receptors found throughout the brain (Barth, Villringer, & Sacher, 2015) (see Figure 1). Some studies suggest that memory is influenced by the relative expression of estrogen receptors as they interact with estradiol (Bean, Ianov, & Foster, 2014). Given the postmenopausal decline in levels of estradiol and potentially beneficial hormones such as progesterone, one hypothesis is that boosting these levels through HT may reduce AD risk in women. Initial support for the protective effects of HT came from observational studies, such as the Kuopio Osteoporosis Risk Factor and Prevention study, which involved a 20-year follow-up of 8,195 women, with 227 cases of incident AD (Imtiaz

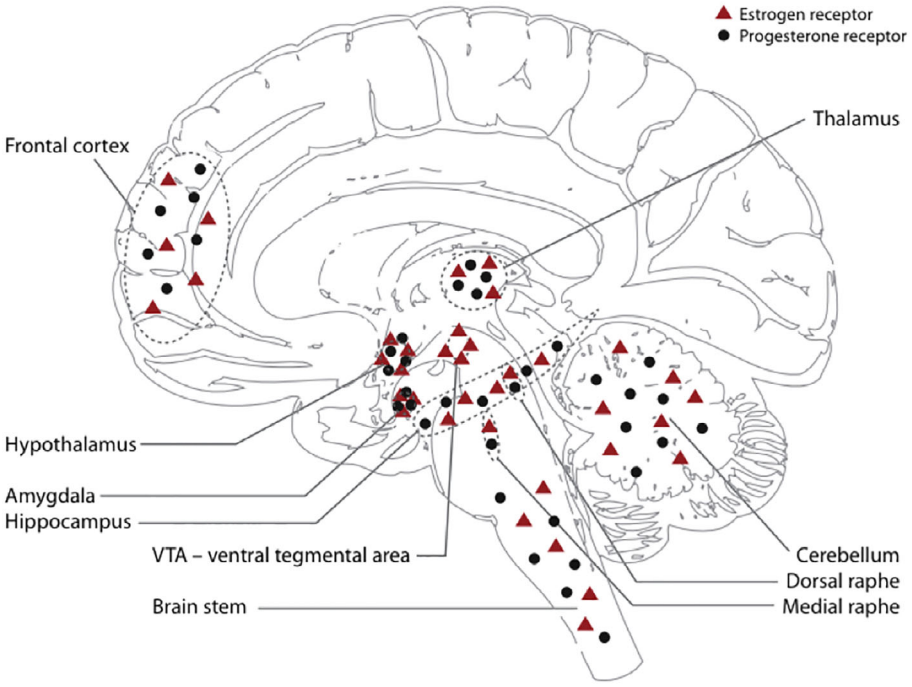

FIGURE 1 Estrogen receptors are found throughout the brain and are predominantly present in the cerebellum, ventral tegmental area (VTA), hippocampus, amygdala, and frontal cortex; as well as in the raphe nuclei of the midbrain (Barth et al., 2015). There is a greater concentration of estrogen-alpha receptors in regions such as the amygdala and hypothalamus whereas estrogen-beta receptors dominate in the hippocampus. More equal representation exists in areas such as the thalamus and the cerebellum (Hedges, Ebner, Meisel, & Mermelstein, 2012; Osterlund & Hurd, 2001). Adapted with permission from Dr. Julia Sacher (Barth et al., 2015)

et al., 2017). In this study, long-term postmenopausal HT was associated with a lower risk of any dementia diagnosis including AD. This contrasts with the aforementioned multicenter trials that randomized women to receive HT and failed to find any benefit of HT on dementia risk (Grady et al., 2002; Shumaker et al., 2003; Shumaker et al., 2004). However, these effects have been recently re-evaluated as additional factors may affect the amount of risk or benefit—such as duration of HT use and the proximity of HT initiation to menopause (Girard, Metereau, Thomas, et al., 2017; Savolainen-Peltonen, Rahkola-Soisalo, Hoti, et al., 2019).

Given the biological complexity of estrogen effects on the brain and AD risk, we tested the following hypothesis: if history of estrogen use is present and protective in older women, this variable may be associated with larger brain volumes, as measured using MRI. Addressing this hypothesis is an important step to understand how HT may influence brain aging and cognitive performance, perhaps motivating an approach to AD risk reduction in clinical practice.

2 | METHODS

2.1 | Participants

The cardiovascular health study (CHS) is a multisite, population-based longitudinal study of coronary heart disease and stroke in individuals 65 and older (Fried et al., 1991). CHS recruitment was based on the Medicare eligibility lists in four communities: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh (Allegheny County), Pennsylvania. In a first wave, 5,201 participants were recruited in the baseline year (1989–1990) of the study. In a second assessment, 687 African–Americans were recruited in year 5 (1992–1993) leading to a cohort of 5,888 participants. The institutional review board at each site approved the study methods, and all participants gave written informed consent.

Participant demographics are shown in Table 1. A separate column identifies, for a particular variable, whether a statistically significant difference ($p < .05$) exists between study sites, based upon one-way ANOVA (continuous) or a chi-squared test (categorical), together with effect size. Differences between sites include variation in socioeconomic and health-related factors, as noted with significant differences for ethnicity, high school completion, diagnosis of AD/MCI and burden of white matter lesions. These factors may contribute to the differences in prevalence of estrogen use across studies (Council TWH, n.d.).

To reduce potential bias, we ran a sensitivity analysis excluding the Forsyth County cohort given their small sample size ($N = 7$) and excluding two subjects whose ethnicities differed from the remaining participant population (neither white nor African American). Significance was unaffected and these records were retained in the full sample.

Of the 562 participants included, APOE4 genotype was available for 528 and 143 of these (27.1%) carried at least one APOE4 haplotype (APOE4 positive). Of these, 10 were homozygous and the remainder were heterozygous. Full methods for obtaining the APOE4 genotypes are reported elsewhere (Kuller et al., 1998).

2.2 | The CHS memory study

By year 4 (1991–1992), 3,608 of the CHS enrollees participated in the CHS Memory Study (CHS-MS) and all had undergone a low-resolution brain MRI scan. In the final year of the study, year 11 (1998–1999), a follow-up high-resolution MRI scan and neurobehavioral evaluations were completed for all available, living participants ($n = 2{,}101$) (Kuller et al., 2003; Riverol, Becker, Lopez, et al., 2015). Due to the late inclusion of the high-resolution 3D T_1-weighted spoiled gradient-recalled echo (SPGR) MRI sequence into the scanning protocol, not all participants had high-resolution anatomical imaging. Here we analyzed brain MRI data from 562 female participants (mean age: 79.4 ± 4.2; range: 71–94 years) who had a high-resolution SPGR MRI scan that met quality control standards.

Neurobehavioral evaluations were made by an Adjudication Committee of experts in dementia who had access to the historical CHS cognitive test scores, primarily the Modified Mini Mental State Examination (3MSE) (Teng & Chui, 1987), Benton Visual Retention Test (BVRT) (Benton, 1945), and the Digital Symbol Substitution Test (DSST) (Salthouse, 1978), as well as the Center for Epidemiologic Studies Depression Scale (CES-D) scores (Irwin, Artin, & Oxman, 1999). Participants were classified as having normal cognition, mild cognitive impairment (MCI), or AD, with specific subtypes of MCI examined in detail only at the Pittsburgh center (Lopez et al., 2003). Dementia classification was based on deficits in performance in two or more cognitive domains that were sufficiently severe to affect activities of daily living and their history of normal intellectual function before the onset of cognitive abnormalities; a memory deficit was not required for the diagnosis of dementia. The committee also reviewed data from vision and hearing tests, history of alcohol intake, activities of daily living questionnaire (Rosano et al., 2005), Informant Questionnaire on the Cognitive Decline of the Elderly (IQ-CODE) (Diesfeldt, 2007), Dementia Questionnaire (Kawas, Segal, Stewart, Corrada, & Thal, 1994), vital status, date of death if relevant, history of hospitalizations, medications to treat dementia, findings from MRI scans, results of neuropsychological assessments, and hospital records (Lopez, Jagust, DeKosky, et al., 2003).

2.3 | Assessment of hormone therapy

HT was assessed annually and included present and when available past estrogen use, "excluding vaginal creams". The decision to exclude this particular type of medication was based on the fact that vaginal cream has local but very little systemic effect due to irregular absorption (Santen, Mirkin, Bernick, & Constantine, 2020). In addition, the use of these creams is typically intermittent. Present estrogen users were defined as women with prescriptions for oral estrogen recorded by medical inventory, regardless of self-reported past use, and this

TABLE 1 Characteristics of the female participants in the CHS Memory Study who had useable MRI in year 11 (1998–1999), by CHS site

	Forsyth County, North Carolina	Sacramento County, California	Washington County, Maryland	Allegheny County, Pennsylvania	Total sample	Study site variance
Number of MRI scans analyzed	7	185	108	262	562	
Age[a]	82.4 (2.6)	79.7 (4.2)	78.9 (4.0)	79.2 (4.3)	79.4 (4.2)	2.24, 0.08 (0.01)
Ethnicity, white[b]	100 (7)	90.8 (168)	98.1 (106)	79 (207)	86.8 (488)	0.00 (0.15)
> High school[b]	42.9 (3)	42.4 (78)	26.9 (29)	45.8 (120)	41.0 (230)	0.01 (0.14)
Diagnosis of AD or MCI[b]	0 (0)	16.8 (31)	19.4 (21)	32.4 (85)	24.4 (137)	0.00 (0.14)
Heart disease[b]	14.3 (1)	24.3 (45)	27.8 (30)	19.8 (52)	22.8 (128)	0.34 (0.08)
Diabetes[b]	0 (0)	5.5 (10)	12 (13)	11.1 (29)	9.3 (52)	0.12 (0.10)
Hypertension[b]	42.9 (3)	51.9 (96)	50 (54)	50 (131)	50.5 (284)	0.95 (0.03)
White matter lesions ≥3[b]	57.1 (4)	31.4 (58)	19.6 (21)	23.1 (60)	25.6 (143)	0.02 (0.13)
Body mass Index[a]	24.1(2.8)	26.4 (4.5)	27.2 (5.0)	27.0 (4.8)	26.8 (4.7)	1.62, 0.19 (0.01)
Physical activity (blocks/daily)[a]	40.0 (45.9)	33.5 (56.8)	27.4 (53.7)	29.1 (38.7)	30.3 (48.3)	0.51, 0.67 (0.00)
Estrogens-(present), year 11, excl vaginal creams[b]	42.9 (3)	26.4 (48)	10.5 (11)	15.2 (38)	18.4 (100)	0.00 (0.18)
Estrogens-(present), baseline, excl vaginal creams[b]	28.6 (2)	24.9 (43)	5.6 (6)	10.9 (24)	14.8 (75)	0.00 (0.22)
Estrogens-(present+past), year 6, excl vaginal creams[b]	42.9 (3)	67 (118)	26.4 (28)	40.6 (104)	46.4 (253)	0.00 (0.28)
Premarin use-(ever), baseline[b]	42.9 (3)	51.6 (82)	19.6 (20)	36.8 (78)	38.1(183)	0.00 (0.24)
Age at menopause[a]	48.0 (13.1)	48.4 (6.4)	46.5 (7.1)	47.5 (5.5)	47.6 (6.3)	2.06, 0.11 (0.01)
Hysterectomy[b]	42.9 (3)	40.8 (69)	37 (40)	38.8 (85)	39.2 (197)	0.93 (0.03)
Oophorectomy[b]	33.3 (2)	30 (48)	27.6 (29)	26.8 (55)	28.2 (134)	0.91 (0.03)
Degrees of freedom *(DF) = 3* for all demographics with exception of ethnicity (*DF = 9*) and diagnosis (*DF = 6*).						

[a]ANOVA: columns 1–4 = Mean (*SD*); last column = *F*-stat, *p*-value (*partial eta-squared* N_p^2: .01 ∼ sm, .06 ∼ med, >.14 ∼ lg).
[b]Chi-squared: columns 1–4 = Yes, %(*n*); last column = *p*-value (*Cramér's* ***phi*** φ_c: .1 ∼ sm, .3 ∼ med, .5 ∼ lg).

information was reported as binary data at each annual time point of the CHS study. Dosage was not made available. Past estrogen users were women responding positively to ever having taken Premarin or other estrogens for hot flashes or other symptoms of menopause and not having a current prescription (Manolio et al., 1993). Past usage was available in data collected from years 4 through 6 (1994–1996) only. Past and present use of Premarin, or CEE's, was reported at baseline (1989–1990) only and was collected in the same way as estrogen use.

For these analyses, we examined present nonspecific estrogen use reported in year 11 (1998–1999) as it corresponds with the year that the high-resolution MRI data were acquired. We evaluated estrogen use from other time points for consistency with results from our main analysis. This included present estrogen use at baseline (1989–1990) as it corresponded with the availability of other relevant data such as age of menopause, hysterectomy and oophorectomy; past and present (combined) HT use from year 6 (1993–1994); and use of Premarin (ever), or CEE's, from baseline (1989–1990) as this was the only CHS study time point in which these data were available.

2.4 | Structural MRI

Brain MRI using the SPGR sequence was completed at each of the four sites using 1.5 T scanners, as detailed elsewhere (Bryan, Manolio, Scertz, et al., 1994). The scanning protocol used in year 11 (1998–1999) included a sagittal T1-weighted localizer sequence, an axial T1-weighted proton-density, and T_2-weighted images. The axial images were 5 mm thick without interslice gaps. White matter hyperintensities, an imaging marker of small vessel ischemic disease, were visually determined using a standardized semi-quantitative 10-point white matter grade (WMG) going from 0 (least) to 10 (most), as described previously (Longstreth et al., 1996). CHS quality control measures included visual review of scans by a neuroradiologist, to ensure that no large space-occupying lesions existed that could hinder analysis (Bryan et al., 1997; Raji, Lopez, Kuller, Carmichael, & Becker, 2009). We also performed our own visual assessment confirming the absence of cropping of brain tissue from the scan field of view and corruption of MR images in the tensor-based morphometry (TBM) image processing stream. For the TBM methods used to process the brain images, refer to Supplementary Data S1.

2.5 | Voxel-wise linear regressions

At each voxel in the brain, a linear regression model (Calabrese, Schneider, Paninski, et al., 2011; Chu, Cui, & Dinov, 2009; Chu & Dinov, 2009) was fit to model relationships between regional brain volumes, our trait of interest and other factors that have demonstrated over time to have an impact on brain structure. Covariates in the analysis included body mass index (BMI) and physical activity as defined and measured in our previous work which identified a significant relationship between these variables and brain volume in a mixed gender superset of the CHS cohort analyzed here (Boyle et al., 2015). Our 16 predictors included: (1–3) site of data acquisition (*dummy variables:* x_1, x_2, x_3), (4) age at year 11 of the study (x_4), (5) ethnicity (*white vs. non-white;* x_5), (6) years of education (*≤/> high school;* x_6), (7-8) clinical diagnosis (*dummy variables:* x_7, x_8), (9) heart disease (x_9), (10) type 2 diabetes mellitus (x_{10}), (11) hypertension (x_{11}), (12) white matter lesions (*</≥ WMG 3;* x_{12}), (13) BMI—year 9 (x_{13}), (14) physical activity—year 10 (*weekly blocks walked;* x_{14}), (15) APOE4 status (x_{15}) and (16) estrogen use—year 11 (x_{16}). We statistically assessed these covariates of interest that predicted volumetric differences across the brain using multiple linear regression:

$$y_i = b_0 + \sum_{k=1}^{K} b_k x_{k,i} \varepsilon_i$$

Here y represents the voxel-wise volumetric measurement, b_o is the y-axis intercept, and b_k represents the regression coefficient for each variable $x_{k,i}$. The b's were estimated using the equation

$$B = inv(X^T X) * X^T Y$$

where B is the column vector of the b coefficients. Subsequent parametric and "p-value" maps were generated to visualize the pattern of voxel-wise model contributions and statistical significance. Then, to control for false positives, we enforced a standard false discovery rate (FDR) correction for multiple statistical comparisons across whole brain voxels using the conventionally accepted false-positive rate of 5% (q = 0.05) (Benjamini & Hochberg, 1995).

After applying this threshold, we further focused our results using an omnibus F-statistic by identifying regions in which the overall model accounted for at least 15% of the total variance. We adhered to this constraint to focus attention on those brain regions where our regression model described an appreciable proportion of variance in our morphological metric of interest. FSL Cluster (Jenkinson, Beckmann, Behrens, et al., 2012) was used to obtain cluster-level statistics for areas of significance in the *F-maps*. Then, to render our results and identify significant regions that correspond to pre-labeled structures in the brain, we aligned our maps with the Talairach atlas and also used a script written in Matlab to convert Montreal Neurological Institute (MNI) coordinates to Talairach coordinates and ensure a more accurate translation.

Student's t-statistical maps were created for each individual variable within the model and were also subjected to p-value thresholding (with *n-k-1* degrees of freedom) against FDR with p = .05 to account for multiple comparisons. Only those significant voxels that were contained within the overall omnibus F-statistic mask were considered further. Brain regions significantly associated with the traits of interest were visualized using these *t-maps* of the beta regression parameters to indicate the direction of change (volumetric expansion or contraction) at each spatial location.

In addition to our main analysis, baseline (1989–1990) estrogen was reviewed in conjunction with relevant variables available only at

baseline such as age of menopause, oophorectomy and hysterectomy status, and the difference between age of menopause and age of beginning HT, or "window of opportunity" (Erickson, Voss, Prakash, Chaddock, & Kramer, 2010).

For exploratory analyses, we used the original regression model with all covariates but stratified the sample by cognitive status to either cognitively normal (n = 425) or cognitively impaired subjects (MCI and AD; n = 137) only. For exploratory analyses involving statistical interactions, we used the entire subject population but added a term to the original regression model, involving the multiplication of two covariates of interest. Interaction terms using current HT (year 11) were modeled to determine whether a particular variable moderated the association of estrogen use with brain volume. Potential confounds included age, BMI, diabetes, hypertension, heart disease, white matter grade and physical activity (PA).

3 | RESULTS

3.1 | Influences on brain structure

Given 16 variables (noted above) and 562 observations, we found our model to be significant with a critical omnibus F-threshold of 6.01, $p < 2.85 \times 10^{-12}$. We present the omnibus F maps for significant

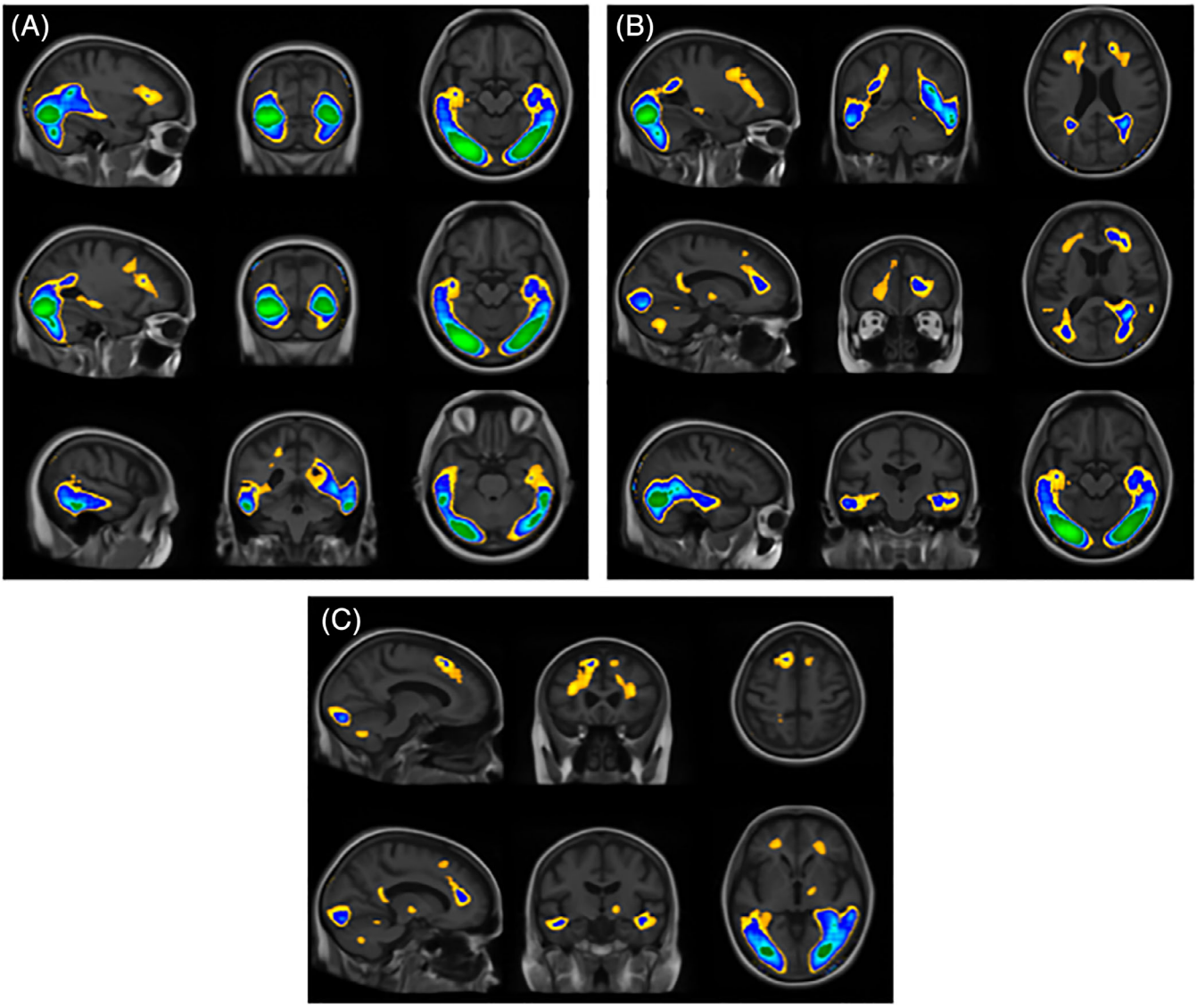

FIGURE 2 Omnibus F-maps visualized using FSL-eyes visualization tool show regional areas where our model as a whole accounted for 15% or greater of the variance. Significant clusters show roughly 15–25% of variance. Slices selected focus on regions with variance ≥25% (*Panel A; green*), 20–25% (*Panel B; blue*), and 15–20% (*Panel C; yellow*). Major clusters of voxels (including max intensities [*F-value*] and related coordinates) were identified using FSL Cluster and regional areas were defined using the Talairach Daemon client (http://talairach.org/daemon.html) as indicated in Table 2

TABLE 2 Cluster and corresponding regional areas defined using the Talairach Daemon client corresponding to the MNI space presented in Figure 2

Panel (Figure 1)	Predominant regions in cluster	F value (max)	Contribution to variance[a]	# Voxels	Peak location (X Y Z)		
A	Right occipital lobe, inferior occipital gyrus, gray matter (bilateral)	22.5	35%	11,068	27	−87	−14
A	Right temporal lobe, fusiform gyrus, gray matter	15.3	27%	249	49	−48	−19
A	Left cerebellum, posterior lobe, tuber	14.2	25%	12	−31	−76	−30
B	Right temporal lobe, sub-gyral, white matter	11.2	21%	121	24	−58	21
B	Right temporal lobe, fusiform gyrus, white matter	10.7	20%	26	46	−34	−17
B	Left limbic lobe, anterior cingulate, gray matter	10.6	20%	4	−16	37	17
C	Right frontal lober, superior frontal gyrus, white matter	9.23	18%	19,194	9	20	48
C	Left sub-thalamic nucleus	8.23	16%	485	−14	−17	2

Note: X,Y,Z = MNI coordinates.
[a]For peak areas of significance.

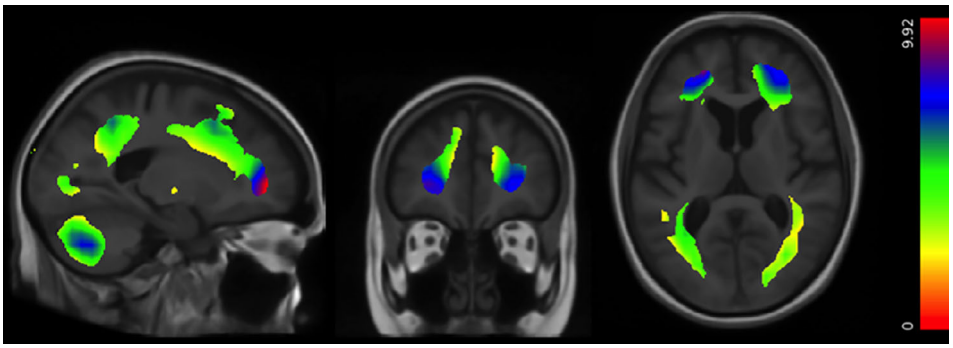

FIGURE 3 Whole brain 3D maps show areas where higher regional brain volume was significantly associated with estrogen usage (reported at time of scan) after adjusting for effects of site, age, sex, ethnicity, educational level, diagnosis, BMI and various cardiovascular disease factors ($N = 562$; $t[546] = 1.96$, $p = .05$, $r = .17$). Beta maps were significant after standard correction for multiple comparisons and represent the estimated degree of tissue excess at each voxel, as a percentage, for estrogen users versus nonusers. There are some areas in the frontal lobe that show ∼10% relatively higher regional volumes for estrogen users, and areas in the parietal and occipital regions which average closer to 5%

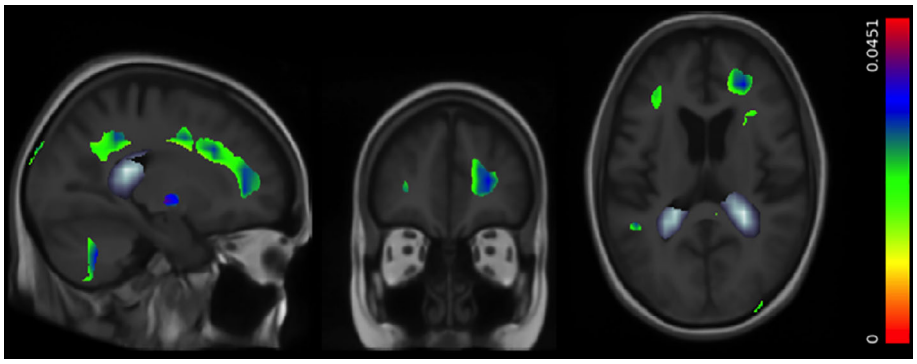

FIGURE 4 Our exploratory analysis shows the surviving significance of physical activity effects despite adjusting for several relevant factors, including estrogen usage. Higher regional brain volume was significantly associated with physical activity (reported as weekly blocks walked; $N = 562$; $t[546] = 1.96$, $p = .05$, $r = .17$). Beta maps were significant after standard correction for multiple comparisons and represent the estimated degree of tissue excess at each voxel, as a percentage, per each additional block walked. With this view we show areas across the brain that extend to ∼.05% for each additional block walked, with the average effects around .025%. The whitish gray areas visible in the posterior horn of the lateral ventricles represent negative values where there was ventricle reduction associated with greater physical activity; ∼ − .1% per weekly block walked

regions of interest (see Figure 2**)** and identify major clusters and local maxima of that particular area of change (above our 15% threshold), regardless of directionality (see Table 2).

We found that our trait of interest—present estrogen use from year 11 (1998–1999), which was closest in time to the high-resolution scan, was associated with significantly higher volume (t[546] = 1.96, p = .05, r = .17) across the whole brain (see Figure 3) even after adjusting for confounding factors such as age and ethnicity. When limited to the statistical constraint of the omnibus F-map, there were no significant areas of gray or white matter volume *loss* in relation to estrogen use in this sample.

For reasons previously stated, other available estrogen measures were evaluated for consistency of results. Analyses of estrogen use prior to MRI, at baseline (1989–1990) and year 6 (1993–1994), and brain volume produced similar results as year 11 (1998–1999; *critical* p = .008) across the whole brain (*baseline, critical* p = .005; *year 6, critical* p = .008). Specifically, past and present Premarin, or CEE, use was also consistent in its association with significantly higher whole brain volume (*critical* p = .003).

Age of menopause, examined both as a continuous and a binary variable (early vs. late menopause), had no independent, statistically detectable effect on brain volume. We were also unable to detect statistically significant effects of the "window of opportunity" on brain volume. We did detect marginally significant positive effects on brain volume with reported bilateral oophorectomy and hysterectomy, respectively, with each predictor showing lower ventricular volume and noticeably increased brain volume in the parietal region (*bilateral oophorectomy, critical* p = .001; *hysterectomy, critical* p = .002). All but one of our study participants who had a hysterectomy also had a bilateral oophorectomy. We were unable to detect effects of HT duration, available only in years 5 (1992–1993) and 6 (1993–1994).

3.2 | Exploratory analyses

For cognitively normal female participants, present use of estrogen in year 11 (1998–1999) remained a significant predictor of brain structure. The positive relationship with estrogen resulted in a critical p = .003 across the whole brain, with notable significance in frontal (p = .004) and parietal (p = .0002) regions. For cognitively impaired participants, estrogen use maintained a significant association with brain volume (*critical* p = .002 across whole brain) with notable significance in the frontal region in concordance with the cognitively normal stratification. These findings suggest that the association between estrogen use and brain volume did not depend on whether a participant was cognitively impaired, and provided reassurance that our analysis was not underpowered to detect such a dependency. To confirm this, we performed a formal test of interaction between estrogen use and diagnosis and found no statistically significant results.

The interaction of BMI and estrogen showed significance (*critical* p = .0006) in overall brain volume and specifically the frontal lobe, suggesting a moderating effect where areas negatively affected by BMI show a larger decrease in volume than BMI alone (see Figure 5).

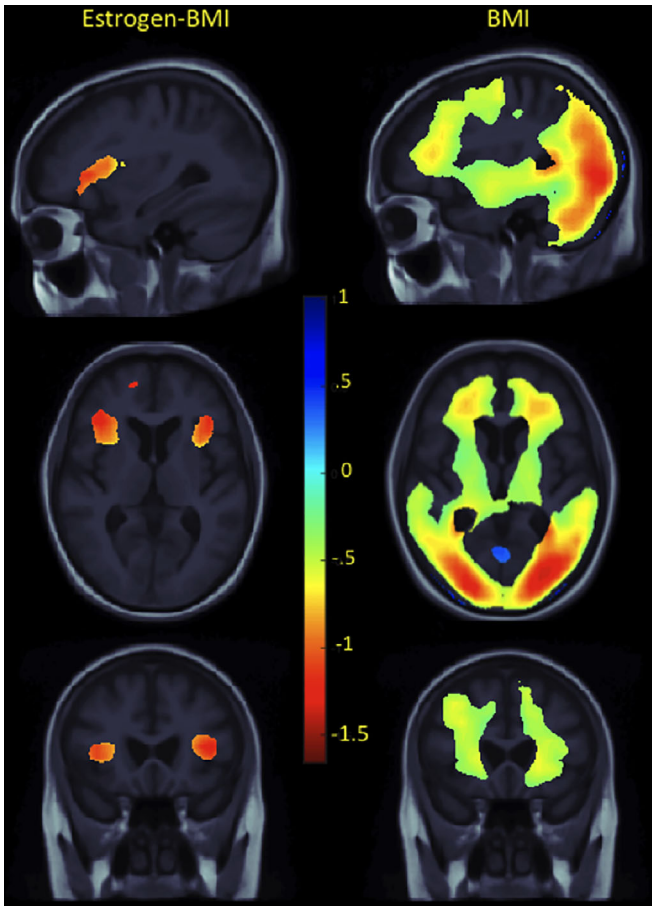

FIGURE 5 Our interaction analysis shows the significance of estrogen-BMI effects after controlling for several factors, including the main effects of BMI and estrogen usage. Here, we present pervasive lower regional brain volume with the main effect of higher BMI (right panel; *N = 548; FDR; critical p = .028*), alongside a larger decrease in volume present in overlapping regions of the frontal lobe with the interactive effect of estrogen-BMI (left panel; *N = 533; FDR; critical p = .0006*). Beta maps were significant after standard correction for multiple comparisons and represent the estimated degree of tissue deficit at each voxel, as a percentage, per unit increase in BMI alone and when combined with HT use. Effects across the brain extend to −1.5% for main effect of BMI with average effects at ∼ − .5%. When combined with HT, effects in frontal regions show average effects at ∼ −1 to −1.5%. Note: Beta maps presented here are not statistically constrained by overall model significance

No other interactions tested were statistically significant indicating the effect of estrogen use on brain structure did not vary significantly as a function of these other variables, including PA. The PA interaction model revealed significance for both HT use and PA independently (*HT, critical* p = .009; *PA, critical* p = .0003, r = .17), with each effect surviving FDR correction. Patterns of relatively greater volume related to estrogen use were consistent with Figure 3. Incidentally, we also include the significance map for PA and brain volume (t[545] = 1.96, p = .05, r = .17) subjected to the statistical constraints of the omnibus map (see Figure 4). Patterns of greater brain tissue volumes and lower ventricular volumes related to PA were consistent with those found in previously published work (Erickson et al., 2010).

In light of recent studies (de Lange, Barth, Kaufmann, et al., 2019) we examined the relationship of APOE4 to brain volume and its possible role as a moderator of estrogen's effect on the brain. Using our predefined model and voxelwise regression, APOE4 positive status alone was associated with marginally significant higher volume (*FDR; critical* $p < .05$) in the ventricles, or ventricular expansion, after adjusting for covariates. We were unable however to confirm the recently reported interactive effect of HT use and APOE4 on brain volume (de Lange et al., 2019).

Finally, as a post hoc test, we tested for mediation effects but did not detect significant mediation for heart disease, hypertension, BMI, or PA as contributing to the relationship between HT and brain volume.

4 | DISCUSSION

The main finding of this paper is that a history of estrogen use in a large cohort of elderly women was associated with larger gray and white matter volumes, in brain regions relevant to cognitive function including frontal, temporal, and parietal lobes. These findings remained statistically significant regardless of time point of estrogen use history, cardiovascular risk factors, genetic make-up or lifestyle factors previously studied such as physical activity (Erickson, Raji, et al., 2010) and obesity (Raji et al., 2010).

These findings are from a relatively large group of women, compared with most prior studies demonstrating a relationship between estrogen and brain structure. Similar to our results, a study of 40 healthy postmenopausal women—of whom 17 were either using or had a history of estrogen use—showed greater gray matter volumes on structural MRI of the frontal, temporal, and parietal lobes including the hippocampus with voxel-based morphometry (Boccardi et al., 2006). A study of 46 subjects (15 men to determine sex-effects and 31 healthy postmenopausal women to determine treatment-effects) showed larger gray matter volumes in the frontal lobes but hippocampal atrophy with increasing estrogen use—which is not in line with our results (Lord, Engert, Lupien, & Pruessner, 2010). Prior work consistent with ours includes a study of 30 women either on or with a history of estrogen replacement therapy that showed use of estrogen and longer duration of use were both correlated with higher frontal, temporal and parietal gray matter volumes compared with women with no history of estrogen use (Erickson et al., 2005). More recent studies show complex results. In a large cohort of 16,000 women from the UK Biobank, machine learning was used to show an association between "brain age" (a composite measure of brain aging derived from MRI) and the use of exogenous estrogen, or HT use, where earlier onset of hormone replacement therapy was associated with less evident brain aging in APOE e4 carriers only (de Lange et al., 2019). Again we were unable to support this finding by showing that APOE4 positive status serves as a positive moderator to HT's effect on the brain. Our analysis was likely underpowered as there were few subjects with both traits.

HT may influence brain structure through several pathways. One possible mechanism might be through genetic or other naturally occurring variation in estrogen receptor expression. Continuous exposure to exogenous estrogen may continue to promote estrogen receptor expression. Genetic variations, including those yet to be discovered, may mediate the differential effects of estrogen on the human female brain. Yet another key factor is timing and duration of estrogen use. Review of multiple prior animal studies suggested a critical window of time in which estrogen use may be beneficial for preserving brain structure and function (Daniel, 2013). Estrogen administered near the time when normal endogenous hormone function ceases was maximally useful for brain health. In contrast, if too much time has elapsed from endogenous estrogen production and exogenous administration, no beneficial effect of estrogen on the brain was observed. Uncertainty remains about how these intervals translate to a human population and what genetic or environmental factors may modulate any such interval (Wang, Mishra, & Brinton, 2020). We were unable to detect a significant effect of such an interval in our study. This may be attributable to the limited availability of the data at baseline (1989–1990), which is not in close proximity to acquisition of the high resolution MRI data obtained in year 11 (1998–1999).

Duration of estrogen usage has also been of interest with studies revealing inconsistencies ranging from cognitive benefits (Erickson et al., 2007) to increased risk for neurodegenerative disorders (Kang, Weuve, & Grodstein, 2004). We were unable to detect effects specific to duration of use; again possibly due to limited data and the timing of data collection relative to the high resolution MRI. Another smaller study found diminishing returns with long-term usage (> 10 years), reporting that higher fitness levels augment the positive effects of shorter durations of hormone treatment and ameliorate the declines associated with prolonged hormone treatment (Erickson et al., 2007). This work supports the interactive effects of estrogen with lifestyle factors such as physical activity and BMI. Our analyses demonstrate an association with the interactive term HTxBMI where interactive effects suggested a greater decrease in volume than with either variable independently. The presence of HT appeared to worsen the negative impact of BMI on brain volume, or the presence of BMI appeared to negate the positive impact of HT on the brain in isolated frontal regions of the brain. Although this is contradictory to some studies (Zsido et al., 2019), excess estrogen present with HT in addition to high BMI may contribute to negative effects on overall health (Cleary & Grossman, 2009) (e.g., breast cancer) including brain health. We did not identify other statistically significant interactions in our model, including age, diabetes, hypertension, heart disease, white matter grade, and physical activity (PA). Still, when examining the interaction of estrogen with PA, the surviving effects of both estrogen and PA in our statistical model reinforce their unique, independent relationships to brain structure. Given the older age range of our cohort, significant atrophy due to aging along with an increase in age-related comorbidities is common. These confounds may be important contributors to the varying effects of estrogen on the brain (Wnuk, Korol, & Erickson, 2012).

Prior animal work suggests that estradiol is the most relevant estrogen for maintaining hippocampal function (Vedder, Bredemann, & McMahon, 2014). Other work suggests that circulating estrogen may

not be as important as previously thought and that local estrogen synthesis and activity within the brain becomes increasingly independent from circulating estrogen following menopause (Li, Cui, & Shen, 2014). This observation may support our inability to find effects of early menopause on brain volume. We did however find association with oophorectomy and hysterectomy status, independently, when added to our model. Each variable showed significantly positive effects on brain volume including a reduction in ventricular volume. These findings may appear to contradict studies that cite a reduction in endogenous estrogen (Rocca, Grossardt, & Shuster, 2010) coupled with negative effects on the brain; however, it is commonly noted that this type of surgery is not detrimental to cognitive health when performed at older ages and may even prove beneficial (Koebele et al., 2019). In mouse models of AD, reduced activity of aromatase—a key enzyme for estrogen synthesis—was related to increased AD pathology (Li et al., 2014). Estrogen may also act to protect or preserve brain structure by maintaining adequate glucose metabolism for as long as 2 years in women randomized to continue estrogen therapy, compared with women randomized to discontinue estrogen therapy (Rasgon et al., 2014). Thus, the ultimate influence of estrogen on the brain is a confluence of complex biochemical processes among which circulating estrogen is only one factor. Estrogen's association with the brain did not vary as a function of cognitive status. To our knowledge, no prior work has simultaneously assessed history of estrogen use on brain structure in both normal cognition and the range of MCI to AD.

The main strengths of this study are its large sample size, high-resolution structural imaging, and multivariate approach. However, interpretation of our results must be tempered, given the complexity of the topic. While past estrogen use was well characterized by self-report, future work should ideally categorize estrogen and its association with brain structure with knowledge of genetic, enzymatic, and quantitative circulating hormonal variables. Such data were not available in our work and results must be interpreted cautiously. Another problem in this study is a strong selection bias for estrogen use and combined estrogen-progestin use. Differences between observational and clinical trials may be attributed to this. Oral estrogens also increase risk of stroke and may have negative effects on brain vascular disease. Women who developed hypertension, venous vascular disease, or transient ischemic attack (TIA) likely stopped estrogen or combined estrogen-progestin prior to entry to CHS. Even so, other work supports the role of estrogen in human cognition and AD risk and such effects are mediated by influences on brain structure as demonstrated here. To understand how estrogen may influence risk for AD, additional work with a randomized design is required. Similar conclusions were suggested based upon the landmark yet controversial results of the Women's Health Initiative (Harman, Naftolin, Brinton, et al., 2005). Despite the complexities, future work to better understand effects of estrogen on the brain may offer new leads for healthy brain aging and AD prevention.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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