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Selective sparing of brain tissue in postmenopausal women receiving hormone replacement therapy

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Abstract

Determining the benefits and/or drawbacks of hormone replacement therapy (HRT) on women's health is an imperative public health goal. Research in rodents suggests benefits of estrogen on neuronal growth and function. However, little research has investigated the effects of HRT on brain tissue in humans. We used high-resolution magnetic resonance imaging and an optimized voxel-based morphometric technique to examine the effects of HRT on brain volume in postmenopausal women. We report two main results: (a) HRT is associated with the sparing of grey matter in prefrontal, parietal, and temporal brain regions and white matter in medial temporal lobe regions, and (b) longer durations of therapy are associated with greater sparing of grey matter tissue. HRT should be considered a possible mediator of age-related neural decline in both grey and white matter tissues.

Keywords: MRI; Voxel-based morphometry; Aging; Prefrontal cortex; Parietal cortex; Hippocampus; Hormone replacement therapy; Estrogen; Temporal cortex

1. Introduction

The effect of hormone replacement therapy (HRT) on the brain health of postmenopausal women is currently being debated. On the one hand, research on non-human animals generated high expectations by showing that estrogen replacement improves functioning of cholinergic neurons [25,27], boosts memory performance on some tasks [22,31], enhances dendritic spine formation [18], and promotes axonal sprouting [26]. In humans, moderate to large benefits of hormone intervention on memory tasks have been observed, and in more than 80% of longitudinal studies women on HRT showed no age-related cognitive decline [40,52]. Despite these encouraging results only a few studies have examined the effect of HRT on human brain tissue.

One of the first studies to examine the effects of estrogen treatment on regional cerebral blood flow (rCBF) reported a series of group by task interactions in the rCBF response when comparing estrogen users to non-users during figural memory and verbal memory tasks [35]. The authors concluded that estrogen treatment positively modulates rCBF activation during memory tasks in regions that are known to subserve memory functions. In another study, conducted longitudinally, estrogen users showed increases in rCBF compared to non-users in some of the same regions affected by Alzheimer's disease (AD) and commonly associated with a memory circuit (hippocampus, parahippocampal gyrus, and middle temporal lobe) [23]. The authors concluded from this study that the longitudinal changes in the estrogen users provide support for a protective view of estrogen on cognition. In corroboration with these findings, a longitudinal functional magnetic resonance imaging (MRI) study with a 3-week estrogen treatment intervention reported a "sharpening of the

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hemisphere encoding/retrieval asymmetry effect" [p. 1197] during performance of verbal and non-verbal working memory tasks and concluded that: "estrogen affects brain organization for memory in postmenopausal women" [p. 1197] [39]. The sum of these functional neuroimaging findings provide preliminary, but encouraging results for the effects of estrogen therapy on brain health and maintenance including establishing the neural correlates of cognitive differences between estrogen users and non-users.

In addition to brain function, some studies have examined the effects of estrogen treatment on the structural integrity of neural tissue in postmenopausal women. At least one study examining the effects of HRT on the volume of the hippocampus has shown greater volumes in estrogen users compared to non-users and men [11]. In addition, two other studies have been conducted that examine the number and extent of white matter hyperintensities in the MRI scans of HRT users compared to non-users [9,38]. One of these studies reported that estrogen users not only performed better on neuropsychological tests of conceptualization and visuopractical skills, but also showed fewer and less extensive white matter hyperintensities than their non-using peers [38]. Another study corroborated this effect by reporting results from a longitudinal investigation that found less progression of subclinical white matter hyperintensities for estrogen users compared to non-users [9]. Finally, a single photon emission computed tomography study examined whether women on estrogen therapy had a greater concentration of cholinergic synaptic terminals. Although they found no differences between users and non-users of estrogen, they did report a positive correlation between duration of estrogen therapy and the concentration of cholinergic synapses in the frontal, parietal, and temporal cortex and anterior and posterior cingulate [49].

Despite these encouraging results several other studies have reported no significant effects of HRT on regional brain volumes [34,35]. For example, one study using automated segmentation algorithms, reported no differences between HRT users and non-users in the overall volume of grey matter, white matter, or CSF despite finding differences in rCBF activation between the groups [35]. In addition, a recent study using manual tracing methods reported that HRT use was not related to larger regional brain volumes in 13 different regions examined in a sample of 42 women (21 taking HRT), although gender related effects were found in a subset of these regions [34]. Another recent study reported larger hippocampi in estrogen users compared to users of selective estrogen receptor modulators, but failed to find a significant difference in hippocampal size between estrogen users and non-users [12]. Finally, a study investigating endogenous levels of estrogen on hippocampal volume and memory performance reported smaller hippocampi and worse memory performance for women with higher levels of circulating estrogens [10]. In addition to these findings, recent reports on large-scale HRT trials have associated combined estrogen–progesterone replacement with an increased risk for dementia [41].

The sum of these recent studies provides equivocal evidence in support of a protective effect of HRT on human brain tissue. However, there are a number of potential reasons for the variable results. For example, to date, studies of estrogen and HRT effects on human brain volume have often focused on a limited number of brain regions [10–12,34,38] despite rodent and monkey research suggesting distributed effects of estrogen on a variety of brain regions including the hippocampus [3,14,18,22,24–28,37], frontal cortex [30,32,33,46,49], parietal cortex [49], basal ganglia [14,49], and amygdala [33]. Therefore, studies examining the effects of HRT on regional brain volume measures may have missed brain regions that are the most affected by HRT use. Along these lines, human studies of the aging brain have more commonly associated structures other than the hippocampus with non-pathological age-related decline [34]. Therefore, if HRT is protective against age-related tissue atrophy, then HRT may exert its effects in regions more commonly associated with age-related changes such as the prefrontal, temporal, and parietal association cortices. However, few of the previous studies have examined cortical regions that are considered most sensitive to age-related decline and only a few of the previously mentioned studies have examined interactions between age and HRT on tissue atrophy measures. In order to circumvent these potential problems, we employed a technique that can examine differences in tissue volume across the entire brain in a point-by-point fashion and use interactions between age and HRT status to determine if estrogen plays a role in sparing tissue affected by non-pathological aging.

Another source of variability among previous in vivo neuroanatomy and neurophysiology studies is the infrequent focus on the effects of duration of HRT treatment on brain volume (however, see [38] and [49]), although such effects of duration have been previously reported in both rodent and human cognitive studies [7,37]. Therefore, variability of HRT duration amongst participants in previous studies may contribute to the variability and inconsistency between results. Here, in a sample of healthy postmenopausal women, we report the first known evidence that HRT may attenuate shrinkage of grey and white matter tissue in regions known for their sensitivity to age-related decline and that the degree of tissue sparing is positively related to the duration of therapy.

2. Methods

2.1. Participants

We examined, in vivo, the brains of 43 postmenopausal women (16 current HRT users, 14 past users—off HRT 1year, and 13 HRT-naive) with high-resolution magnetic resonance (MR) imaging. Our target participant pool was right-handed, high functioning, community-dwelling older adults, who were at least 55 years of age, and were recruited from newspaper ads, public flyers, and campus-wide e-mailings.

Participants were excluded from the study if they were below 55 years of age, scored below 51 on the Modified Mini-Mental State Examination [45], or had a known history of stroke or other organic brain dysfunction. Although we screened for disease and dementia, we cannot rule out the possibility that some members of our sample were in a preclinical disease state, but because of the healthy demographic characteristics and MMSE screening procedure we will refer to our population as 'non-pathological'. Additionally, for safety concerns related to a magnetic resonance imaging setting, participants were excluded if they had metallic implants, pacemakers, or reported claustrophobia prior to, or during, the assessment. The Institutional Review Board of the University of Illinois approved this research project, and all relevant ethical standards of human subject treatment were met or exceeded. All participants provided written informed consent before participation.

The descriptive statistics for demographic variables, including the type of, and duration of HRT, gravidity (number of pregnanices), parity (number of births), socioeconomic status (SES), and Kaufmann Brief Intelligence Scale (K-BIT) scores are presented in Table 1. SES was determined by asking participants which taxable income bracket they fell into (if single (1) <30,000, (2) 30–60,000, (3) 60–135,000, (4) >135,000, if married (1) <45,000, (2) 45–100,000, (3) 100–160,000, (4) >160,000). We collected information on gravidity and parity because a few recent studies have suggested a relationship between cognitive decline and nulliparity and late menopause [29]. In the HRT groups, 23 of 30 women were on an unopposed estrogen therapy (Premarin) while seven were taking estrogen opposed by progestin (Prempro).

Because of the small number of participants in each of the groups, the distribution of the samples may not conform to a normal distribution. Therefore, we performed Kruskal–Wallis nonparametric tests on each of the demographic variables except for duration of treatment. To test if duration of treatment significantly differed between current and prior users we performed a nonparametric two-sample Kolmogorov–Smirnov (K–S) test. All nonparametric tests were thresholded at $p < 0.05$. In addition, Spearman rank correlation coefficients were obtained between all variables and thresholded at $p < 0.05$.

2.2. Measures

Our primary outcome measures were based on standardized grey and white matter maps, rendered from high-resolution magnetic resonance images. For 26 participants, high-resolution (0.98 mm × 0.98 mm × 1.30 mm) brain images were acquired using a 3D spoiled gradient (SPGR) sequence on a 1.5 T MR scanner (General Electric, Milwaukee, WI). For the remaining 17 participants, high-resolution (0.98 mm × 0.98 mm × 1.30 mm) brain images were acquired using an MPRAGE gradient sequence on a 3 T MR scanner (Siemens Allegra, Germany).

We used an optimized voxel-based morphometric (VBM) technique [1,15,16] to assess the impact of hormone replacement therapy, age, and duration of therapy on differences in the volume of grey matter and white matter. Multiple studies have now used this technique to examine differences in brain tissue volume in Alzheimer's disease [5,6,17,21,47], Parkinson's disease [5], and normal aging [8,16]. The optimized-VBM technique provides a means to estimate tissue atrophy in a voxel-wise fashion throughout the brain with reasonably high spatial resolution. This allows regionally specific conclusions about the variables of interest on changes in brain matter, and represents a significant advantage over measures of global atrophy in estimating brain integrity. This technique has gained popularity over the past few years and has contributed to a large number of both clinical and methodological studies (e.g. [1,4–6]). The optimized approach employed in this report is similar to other recently published studies that have examined the effects of aging and pathological disturbances on the brain except for the following changes: (a) we employed FSL instead of SPM to conduct our analyses; (b) we used affine transformations for all registrations instead of using a combination of affine and nonlinear transformations.

To reduce potential registration bias due to differences in brain structure between groups [1,4,15,16] we initially

Table 1
The mean, standard deviation, and range of demographic information for current, past, and never users of HRT

	Current	Past	Never
N	16	14	13
Age	68.9 (5.5), range: 60–79	66.2 (6.2), range: 58–78	68.4 (5.5), range: 57–76
Education	16.1 (3.1), range: 12–22	16.6 (2.9), range: 12–24	15.8 (2.7), range: 11–20
SES	1.3 (.96), range: 0–3	1.4 (.83), range: 0–3	1.2 (.83), range: 0–3
K-BIT	114.8(6.4), range: 101–121	114.3 (7.5), range: 103–120	118.3 (6.3), range: 107–128
Parity (# births)	2.8 (1.3), range: 0–5	2.9 (2.8), range: 0–10	2.5 (1.8), range: 0–6
Gravidity (# pregnancies)	3.2 (1.6), range: 0–6	3.2 (2.9), range: 0–10	3.2 (2.6), range: 0–8
Age at menses	12.5 (1.1), range: 11–15	12.4 (.83), range: 11–14	11.8 (1.3), range: 10–14
Age at menopause	45.6 (6.3), range: 32–55	48.0 (5.8), range: 34–57	46.2 (5.6), range: 39–56
Type of therapy	10 Premarin	13 Premarin	X
Duration of therapy	13 years (4.5), range: 2–20	11 years (3.1), range: 1–18	X

Standard deviations are represented in parentheses. There was no statistical relationship between any variables and use of HRT using nonparametric Kruskal–Wallis and K–S tests.

constructed a sample-specific stereotaxic template. First, we removed all non-brain matter using an accurate and robust deformable model algorithm [43]. The skull-stripped image for each participant was then spatially registered to stereotaxic space (152 T1 MNI, Montreal Neurological Institute) using a robust 12-parameter affine transform [20]. Next, we averaged these normalized images to create a representative composite, and then smoothed the composite image with an 8 mm (full-width at half-maximum) 3D Gaussian kernel [1,15,16,21,48].

The next step was to create the prior probability maps that would seed the final segmentation. To do this, each skull-stripped image in native space was re-registered to MNI space using the template as the reference image [1,15,16,17]. This registration stage will be referred to as the second level registration. Next, prior probability tissue density maps were obtained by segmenting the skull-stripped images into three separate 3D maps representing the probability density of the cerebrospinal fluid, grey matter, and white matter, at each point in the MR image for each participant, using a well-established automated segmentation algorithm [53] that requires minimal user intervention. The resulting 3D maps for each tissue type were then registered to the template image by applying each participant's transformation matrix from the second level registration to their probability density maps. These normalized and segmented probability maps were then averaged and smoothed with a 12 mm kernel and subsequently used as prior probability maps to seed the final segmentation.

For the final segmentation, the original skull-stripped images were re-segmented into 3D maps of grey matter, white matter, and cerebrospinal fluid using the prior probability density maps as seeds for the segmentation. A priori seeding of the segmentation algorithm has been shown to increase both robustness and precision of the resulting segmented maps, particularly for deep grey matter structures [1,15,16]. These grey matter, white matter, and cerebrospinal fluid probability maps were then registered to the template image by applying the transformation from the second level registration to each image. These final normalized grey matter, white matter, and cerebrospinal fluid probability maps were spatially smoothed with an 8 mm full-width at half-maximum Gaussian kernel to satisfy the requirements of the random field theory upon which the final analyses would be based, and then forwarded to the analysis stage.

In order to preserve information about changes in voxel volume due to spatial registration, we computed the Jacobian determinant of the second level transformation matrix. The Jacobian determinant of the spatial transform matrix can be viewed as an index of the amount of volumetric compression that each voxel is subjected to when stretching, shearing and compressing the images into stereotaxic space, thus representing a modulating factor that can be used to preserve volumetric information at each voxel in the image [1,15,16]. Given that our transformations into stereotaxic space were constrained to linear modulations, the amount of compression at each voxel was uniform throughout the head, and thus we entered the Jacobian determinant of the spatial transformation matrix for each participant as covariates in our statistical model.

2.3. Analysis

To assess the effects of HRT, age and HRT duration on regional brain volume, we examined each tissue map for systematic variation associated with the participant's HRT status, age and duration of treatment. Simply stated, each point in the CSF, grey and white probability density maps for each participant was entered into a separate multiple regression with the participant's HRT status, age, and duration of HRT treatment as independent variables of interest. In order to control for any confounding effects of SES, education, or differences in spatial transformations on differential tissue volume between HRT users and non-users, participant's SES, education, and Jacobian determinants were considered covariates. The resulting analysis yielded four statistical parametric maps of interest for each tissue type: one representing the change in tissue volume at each point in the brain associated with HRT use (main effect of HRT), one representing changes due to age (main effect of age), one representing changes associated with length of treatment (main effect of duration), and one representing the degree to which HRT moderated the change in tissue volume associated with age (the age × HRT interaction). Our images were rendered using a minimum *Z*-score of 4.0 uncorrected for multiple comparisons. Similar thresholds have been used in other VBM studies to control for small sample sizes and multiple comparisons [5,6,21].

Because of the high number of multiple comparisons in a voxel-wise analysis it is sometimes preferable to perform a region-of-interest analysis on smaller brain structures, defined a priori, in which effects may be masked by stringent thresholding criteria in the whole-head analysis. Since animal studies have reported robust effects of estrogen on the hippocampus but human studies have reported mixed results, we conducted an ROI analysis of the anterior portion of the hippocampus, which would provide additional information regarding the effects of estrogen treatment, duration of treatment, and age on the volume of the hippocampus. Therefore, the anterior hippocampus was examined further by using a region-of-interest approach using an ROI template. Because of the smaller number of multiple comparisons and fewer voxels overall in an ROI analysis, lower statistical thresholds are usually preferred. Therefore, we applied an uncorrected minimum voxel-wise *Z*-score of 2.33 ($p < 0.01$) to the hippocampal ROI.

3. Results

3.1. Demographic variables

We tested for differences in the demographic variables between the three groups by using the nonparametric

Kruskal–Wallis test. We found non-significant effects of age ($\chi^2 = 2.82$; $p = 0.245$), education ($\chi^2 = 0.108$; $p = 0.948$), SES ($\chi^2 = 0.577$; $p = 0.749$), age at menses ($\chi^2 = 2.71$; $p = 0.257$), age at menopause ($\chi^2 = 1.99$; $p = 0.369$), gravidity ($\chi^2 = 0.898$; $p = 0.638$), parity ($\chi^2 = 1.01$; $p = 0.603$), and K-BIT ($\chi^2 = 3.1$; $p = 0.210$) between the three groups. In addition, the two-sample K–S test revealed a non-significant difference for duration of hormone use between current and prior users ($Z = 0.371$; $p = 0.999$). We also conducted a series of correlations between all of our variables and found only one significant correlation ($p < 0.05$) between the number of births and number of pregnancies ($\rho = 0.88$). The lack of group differences on any of the demographic or cognitive variables decreases the likelihood that our results are confounded by any of these characteristics.

3.2. Magnet effects

In order to assess any potential confounds with respect to the strength of the two magnets, we directly compared the grey and white matter maps from the 1.5 T to those from the 3 T magnet. We found no significant effect of scanner or any interaction with scanner with any of our variables of interest anywhere in grey matter or white matter, even at a less stringent voxel-wise threshold ($p < 0.05$).

3.3. Grey matter

We found no significant differences in grey matter volumes between current and past users of HRT. However, a comparison of HRT users (regardless of group) and HRT-naïve women revealed a significantly greater amount of grey matter tissue in HRT recipients than in non-users. This effect was largest throughout the frontal, prefrontal, and temporal regions (Fig. 1a). Moreover, we found that HRT had significantly greater effects with increasing age (HRT × age), indicating that the sparing of brain tissue in HRT users may ameliorate age-related threats to brain integrity (Fig. 1e). Thus, our findings buttress the hypothesis that HRT effects on brain tissue are age-specific as suggested by non-human animal [37] and human cognitive [7] studies.

Although non-human animal studies have found effects of estrogen on basal ganglia and subcortical structures we failed to find any effect of HRT status on these regions. Although an ROI analysis of these structures was not conducted in this study, it remains a possibility that any changes in subcortical structures were undetectable in our whole-head analysis. Therefore, a ROI-based analysis of these regions may be a more appropriate method to assess the impact of hormones. Future research is warranted to address this possibility.

We observed a robust effect of HRT duration on grey matter, with greater regional sparing associated with longer

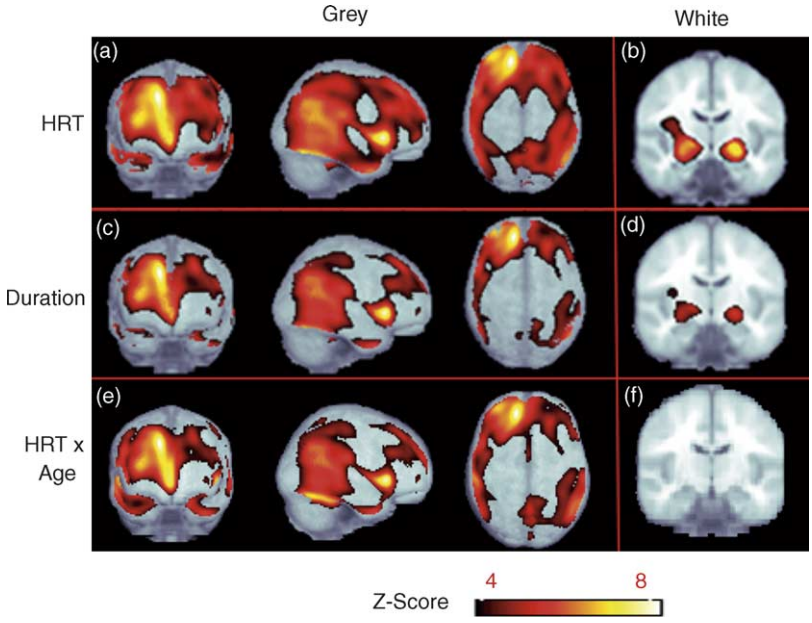

Fig. 1. (a) The sparing effect of HRT on grey matter. The effects extended from prefrontal regions (superior, middle, and inferior frontal gyri) to medial frontal regions (medial frontal and anterior cingulate gyri) as well as parietal (superior and inferior parietal lobules) and temporal lobe (medial and inferior temporal gyri). (b) The sparing effect of HRT on white matter was localized to the medial temporal lobe immediately superior to the hippocampus. (c) The sparing effects of duration of therapy on grey matter. It is notable that the effects of duration were in the same regions as the general effects of HRT treatment, except to a lesser degree. (d) The sparing of white matter was observed in the same region as that of the main effect for HRT except to a lesser degree. (e). The effect of the interaction between HRT × age. Notably, the grey matter regions that were found to be significant in this interaction were highly overlapping with the regions where we found main effects of HRT and duration. (f) The white matter effects for the HRT × age interaction were absent in this sample. In all images neurological convention is used (left is on the left). All images were thresholded at a Z-score of 4.00.

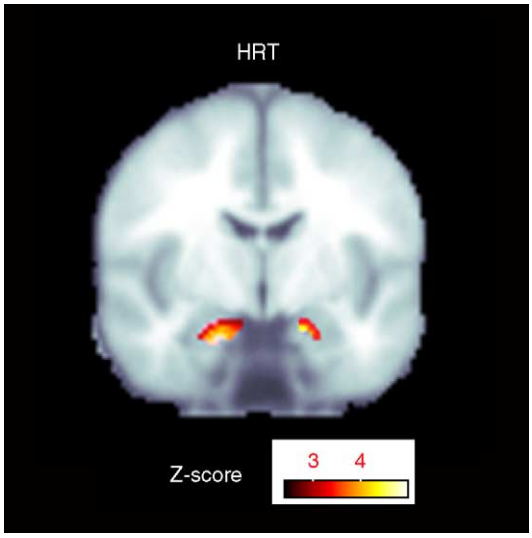

Fig. 2. The sparing effect of HRT on the hippocampus in a region-of-interest analysis. The image is presented in neurological convention (left is on the left). No effects of duration of treatment were found in the hippocampus. The map has a *Z*-score threshold of 2.33.

durations of therapy. Again, the effect was evident in age-sensitive regions of the brain: the prefrontal, parietal, and temporal association cortices (Fig. 1c).

We further examined the grey matter of the hippocampus in a region-of-interest analysis. That analysis revealed greater anterior hippocampal volume in HRT users (Fig. 2) than non-users. A similar effect was found for the age × HRT interaction in the left anterior hippocampus (Fig. 3). No effect was found for duration of treatment.

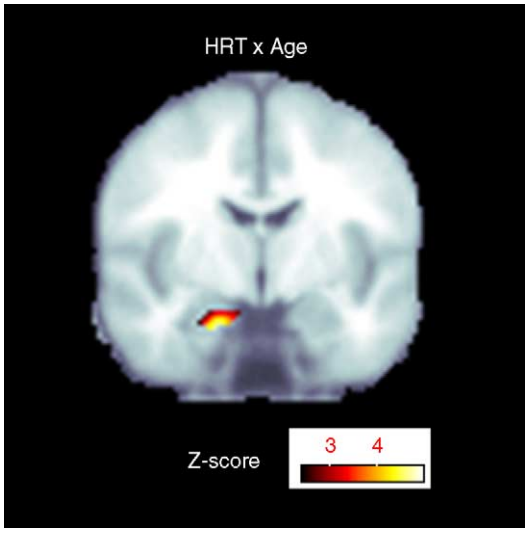

Fig. 3. The sparing effect of HRT with increasing age (HRT × age) on the hippocampus in a region-of-interest analysis. The image is presented in neurological convention (left is on the left). The map has a *Z*-score threshold of 2.33.

3.4. White matter

There were no differences in white matter volume between current and past users of HRT. However, we found HRT-related sparing of white matter when comparing HRT use (regardless of group) to naïve users (Fig. 1b). This effect was primarily limited to the medial temporal lobe, superior to the hippocampus. The benefit of prolonged therapy was also observed in that region (Fig. 1d). We found no significant interaction between HRT and age on white matter (Fig. 1f).

3.5. Cerebrospinal fluid

Additionally, we found a greater volume of cerebrospinal fluid in women who were not taking HRT. The effects of CSF were largely localized to the lateral ventricles as well as a strip along the perimeter of the brain. Notably, one area where we found increased CSF was along the temporal horn of the lateral ventricle near the hippocampus. This result supports our finding that HRT protects against medial temporal lobe atrophy.

3.6. Opposed versus unopposed therapy

We were unable to formally examine the differences between opposed versus unopposed estrogen users due to the small number of women on opposed estrogen therapy in our sample. However, the removal of data from the seven women on opposed estrogen therapy from the statistical model had no significant effect on our results.

4. Discussion

Notably, the areas where HRT had its largest effects were similar to the regions known for increased age-related vulnerability: prefrontal, parietal, and temporal association cortices. Importantly, these regions are also thought to support cognitive functions such as verbal memory, which is also positively influenced by HRT [40,52]. The significant age × HRT interaction suggests that the effect of HRT on grey matter reduces the decline in tissue volume associated with aging. Therefore, our results support the belief that estrogen positively affects the structural integrity of aging brain tissue.

Our results converge with previous human research showing a benefit of estrogen on brain structure and function as well as reduced AD risk associated with HRT [7,11,18,22,25,27,31,37,39,44,51,52] as well as with findings that suggest sparing effects of HRT treatment on rodent and human hippocampus [7,18,25–27,40,51,52]. Moreover, the HRT-related differences in anterior hippocampus in the current study is in accord with the reported association between anterior hippocampal volume and memory in older adults [19] and is also consistent with protection of hippocampal structure and function in non-human animals on estrogen replacement [18,25,27]. Cortical and hippocampal

deterioration in non-pathological aging and more severe atrophy in these structures in certain pathologies suggests a potential mechanism by which some aspects of atrophy, pathological or otherwise, may be mediated through the use of a hormone intervention. How, whether, and under what circumstances this is possible should be examined further.

The sum of these findings argues that estrogen may play a protective or sparing role on brain tissue in certain cortical regions in non-pathological aging and that the effects may be largest in those regions most sensitive to age-related declines in tissue volume. Moreover, our findings beg the question of whether similar effects would be seen in pre-clinical populations with greater declines in regional brain volume, or women with the APOE-e4 allele.

We also found it interesting that the effects in white-matter were directly superior to the hippocampus. The white matter effects extended from the hippocampus toward the basal ganglia. Although we do not report effects of hormones on the basal ganglia, rodent research has commonly implicated this region in estrogen research [22] and the relationship between the hippocampus and basal ganglia are well-documented [22,31]. Therefore, it seems probable that the white matter effects are related to both of these structures. An ROI analysis of the basal ganglia regions and a correlation with hippocampal atrophy measures may prove fruitful in addressing this question. The white matter effects might also be related to the hippocampus such that hippocampal atrophy is accompanied by deterioration of its efferent and/or afferent white matter tracts. Diffusion tensor imaging is one method that may be able to test this possibility.

A positive relationship between duration of HRT use and the degree of tissue sparing is also in accord with some studies that report a positive relationship between duration of treatment and the number of cholinergic synapses in certain cortical regions and an inverse relationship between duration of treatment and the total number of white matter hyperintensities [38,44]. In addition, in our study, the main effect of HRT on medial temporal white matter and the incremental effect of duration are consistent with recent findings of an inverse link between risk for AD and duration of HRT in a sample of postmenopausal women [51]. However, publications in non-human animals suggest a limited time window and a limited duration for positive effects of estrogen [24]. It may be possible that the optimal duration of HRT use is longer in humans than rodent models suggest, but that the duration of therapy in humans beyond a certain length reduces the efficacy of the treatment. However, as our results show that the benefits of estrogen treatment are strongest with extended therapy regimen, it becomes clear that studies based on short-term interventions in humans could have overlooked the potential benefits of HRT. More research should be conducted to examine the relationship between duration of treatment, cognitive and neural protection and optimal time windows for the efficacy of treatment.

In animal models the density of estrogen receptors (ERα and ERβ) differs by region, with the prefrontal cortex being especially high in ERβ [27]. Thus, although estrogen is known to act on regions lacking estrogen receptors (e.g. striatum), the action of HRT on specific brain regions due to differential changes in estrogen receptors with age is possible. However, non-genomic mechanisms of estrogen such as angiogenesis or regulation of neurotransmitters may also contribute to the sparing of tissue with HRT use. In short, the mechanisms underlying estrogen's effects on neural tissue are complex and there may be multiple paths by which estrogen acts.

It is interesting to consider our results in relation to the recent results from the Women's Health Initiative (WHI). The WHI has reported an increased risk of dementia and lower MMSE scores associated with combined estrogen and progestin treatment [41] and unopposed estrogen treatment [13,42]. Although we do not have any behavioral or cognitive data to relate to our volume measures, the sparing effect of HRT with increasing age suggests that the effects of HRT on grey and white matter tissue is not detrimental as might be inferred from the WHI study. However, numerous differences exist between this study and the studies from the WHI that could contribute to the seemingly discrepant findings. First, although recent publications from the WHI report that both combined estrogen and progestin as well as unopposed estrogen have deleterious effects on MMSE scores and dementia, it remains a possibility that unopposed estrogen has different effects on neural tissue than opposed estrogen plus progestin therapy. Our sample consisted mostly of women taking unopposed estrogen. We were unable to formally address the question of whether opposed estrogen therapy differs from the outcome of unopposed estrogen therapy on brain health, but the removal of those subjects from our sample did not change the pattern of results. In any case, it still remains a possibility that opposed estrogen has a different effect on the neural tissue of women than unopposed estrogen. More research is warranted to examine this issue.

In addition, other differences could exist between the current study and the studies conducted through the WHI including duration of treatment (our study has a longer mean duration than the mean duration of the WHI), age starting treatment, severity of menopausal symptoms, etc. which may play a role in determining the efficacy and benefit of HRT on brain tissue. Some of these variables may prove to be determining factors in the variability currently seen among studies.

It is also interesting to consider the effects of HRT in the context of previous findings on the relationship between aerobic fitness and brain structure in aging humans [8]. In both samples, the greatest age differences were observed in the prefrontal, parietal, and temporal grey matter, and therein lied the greatest benefits of the respective interventions. This similarity may reflect the fact that estrogen increases some of the same neurochemical and molecular markers as those often implicated in exercise related neuronal changes [3,14]. Estrogen is also known to positively affect physical activity levels, but the relationship between estrogen and physical fitness on human brain tissue and neuropsychological

functioning remain unanswered. However, these findings, in combination, suggest the possibility of a common pathway for brain health maintenance in older populations.

Although VBM is thought to provide a very robust and spatially precise method for investigating the integrity of brain tissue, there are caveats relevant to the technique that currently limit the applicability and interpretations of VBM results. For example, the registration routines that are the most optimal, robust, and valid for drawing conclusions about the volume of the underlying tissue being registered, continue to be a source of debate [1,2,4]. Some studies suggest that different registration parameters and methods may be applicable under certain circumstances and not others, depending on the regions of interest or the population of interest [36,50]. However, the majority of VBM studies that have examined aged individuals or populations with neuropathologies have followed the optimized and modulated VBM protocol similar to those developed by Ashburner and Friston [1,2] and Good et al. [15], and used in the current study. Furthermore, although methods of registration can produce errors, techniques such as manual tracing that do not rely on registration are also prone to error [47]. Recent studies examining the consistency between VBM and manual tracing report both some overlap and some discrepancy between the two methods [17,47]. Although beyond the scope of this paper, further research examining the association between VBM and manual tracing should be conducted to further enlighten the commonalities and discrepancies between the two methods.

The findings reported here should be interpreted in the context of two limitations. First, the cross-sectional design of this study does not allow for the examination of a causal relationship between HRT and differences in brain tissue. Second, our findings are limited primarily to unopposed estrogen replacement therapy, and do not necessarily generalize to an opposed estrogen replacement regimen. Nonetheless, the important implication of our findings is that the effects of HRT, beneficial or otherwise must be examined with attention to multiple specific factors such as type and duration of therapy, participants' age and specific brain locations that may be the most likely targets of intervention.

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