

Preliminary evidence that estrogen protects against age-related hippocampal atrophy

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Abstract

Few studies have examined gender differences in hippocampal volumes, and the potential effect of estrogen on these measures has not been well studied. We used MRI to measure hippocampal volumes in elderly Mexican American men and women subjects in order to determine if there were gender differences and if estrogen replacement therapy (ERT) had an effect on hippocampal volume in postmenopausal women. MRI measures of hippocampal volumes (normalized to intracranial volume) were compared in 59 women and 38 men. Further comparisons were made between men subjects, women subjects taking ERT, and women subjects not taking ERT. There were no significant effects of gender on normalized hippocampal volumes. However, women subjects taking ERT had larger right hippocampal volumes than women subjects not taking ERT and larger anterior hippocampal volumes than men subjects and women subjects not taking ERT. These findings suggest a neuroprotective effect of estrogen.

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1. Introduction

A variety of studies in both humans and animals support the biological credibility of estrogen as a neuroprotective agent with potential effects on the pathogenesis of Alzheimer's disease (AD). Epidemiological observations that postmenopausal women taking estrogen replacement therapy (ERT) have a lower prevalence of AD sparked considerable interest in the possible protective effects of this hormone [21,41,56]. A number of studies have examined cognitive performance, particularly memory performance, in postmenopausal women, and have reported a beneficial effect of ERT [29,31,46–48]. While there are some conflicting findings, especially with regard to the use of estrogen for the treatment of AD, a growing body of evidence indicates that ERT reduces the risk of AD in postmenopausal women [1,2,5,7,21,23,32,41,56].

A number of studies have shown that functional and structural brain changes occur prior to the onset of AD and may be used to identify those at risk for the disease. MRI measures of hippocampal atrophy have been shown

to be good markers of both AD and presymptomatic AD [9,27,28,34,35]. Older subjects with poor memory performance, often termed mild cognitive impairment (MCI), are also at risk for AD, and their impaired memory appears to be linked to hippocampal atrophy. Both MCI subjects and subjects genetically at risk for AD show reduced hippocampal volumes in studies using both MRI and CT [9,17,51,58], and a recent MRI study reported that reduced hippocampal volume was a good predictor of subsequent conversion to AD in MCI subjects [28]. Reduced blood flow and glucose utilization in the temporoparietal neocortex, as shown with both positron emission tomography (PET) [10,14–16] and single photon emission computed tomography (SPECT) [6,12,43] have also been reported in subjects with AD [11,42,52], and reduced glucose metabolism in the posterior cingulate gyrus may be a sensitive marker of early, and perhaps presymptomatic AD [37]. These neuroimaging findings have resulted in the use of reduced hippocampal volume and temporoparietal and posterior cingulate hypometabolism as biological markers of AD and presymptomatic AD. If low estrogen is indeed a risk factor for the development of dementia, we would expect to see similar PET and MR markers of AD in women with low estrogen levels.

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A few neuroimaging studies have shown effects of estrogen on brain function [13,46,49]. Both PET and fMRI studies have shown different activation patterns in postmenopausal women taking estrogen and postmenopausal women not taking estrogen, even though estrogen was not associated with enhanced task performance [46,49]. Using PET and the metabolic tracer, FDG, we recently found that women not receiving ERT had glucose metabolic ratios that were intermediate to those of women receiving ERT and AD patients even though the women not taking ERT showed no signs of cognitive impairment [13]. These kinds of findings suggest that neuroimaging may be particularly sensitive to estrogen-mediated effects on brain function, and suggest that neuroimaging markers of AD and pre-clinical AD may be useful for evaluating the association between estrogen use and risk of AD.

If estrogen is associated with the development of dementia, we would predict reduced hippocampal volumes in women with low estrogen levels, perhaps even in the absence of cognitive impairment. While hippocampal volume is reportedly negatively correlated with age in healthy elderly subjects [26,33] there are conflicting findings regarding age-related gender differences in hippocampal atrophy [26,40,44,54] and it is not clear what role, if any, ERT plays. We used MRI to evaluate the effects of gender and estrogen use on hippocampal volume in elderly men and women with the hypothesis that hippocampal atrophy would be attenuated in women taking estrogen.

2. Methods

Subjects for this study were chosen from the Sacramento Area Latino Study of Aging (SALSA), an epidemiological, population-based study of community-dwelling elderly Mexican American individuals. Of the 1789 subjects in this study, 122 received MRI scans, including 52 men and 70 women as part of a sub-study to examine brain structure in normal, cognitively impaired and demented subjects. All subjects received a battery of neuropsychological tests, including the modified mini-mental status exam (3MS), a modified version of the MMSE designed to sample a broader range of global cognitive ability [57]. Through a series of steps, cognitively impaired subjects were evaluated for dementia. All subjects receiving MRI were categorized as normal, cognitively impaired or demented. Subjects with dementia were excluded from this analysis. The cognitively impaired subjects performed poorly on one or more neuropsychological tests and subsequently underwent a neurological examination. After examination, these cases went to case adjudication and did not meet criteria for dementia. Operational criteria for dementia required clinically significant impairment in two or more cognitive domains and clinically significant impairment of independent functioning. Collection of data on estrogen use was obtained through interviews with the subjects and verified by exam-

ination of the prescriptions for all current medications. The study included 46 women not taking estrogen (ERT–), 13 women taking estrogen (ERT+), and 38 men.

2.1. MR data acquisition and analysis

MR scans were acquired on a 1.5T GE Signa Horizon LX NV/I (neuro-optimized) system using a coronal 3D spoiled gradient recalled echo (IR-prepped SPGR) T1-weighted acquisition protocol (TE 1.9 ms, flip angle 20°, fov 24 cm, matrix 256 × 256, 124 contiguous slices, slice thickness 1.6 mm). Hippocampal volumes were drawn by a single rater blind to subject classification using an in-house program to create a volume out of the areas traced on contiguous slices [36]. Volumes for a subset of subjects were drawn twice by two raters in order to determine inter- and intra-rater reliability using intraclass correlations.

First, the MR dataset was resliced, using the sagittal view to align the coronal data set perpendicular to the long axis of the left hippocampal formation. The boundaries of the hippocampus were manually traced on contiguous 1.6 mm coronal slices in the anterior to posterior direction. While the boundaries of the hippocampus were traced on coronal slices, the corresponding sagittal and axial planes for any point on the coronal image could also be viewed (Fig. 1).

Within the term “hippocampus” we include the dentate gyrus, the hippocampus proper (CA3, CA2, and CA1), the subiculum, and the pre- and parasubiculum. The entorhinal cortex is not included in our measurements. The boundaries of the hippocampus in each subject were identified using anatomical landmarks. The rostral pole of the hippocampus was identified in coronal and sagittal images (Fig. 1). Typically, the temporal horn of the lateral ventricle surrounded the lateral and dorsolateral portions of the hippocampus at this level. The hippocampus could also be differentiated from the overlying amygdala by the white matter of the alveus that surrounded the hippocampus.

At rostral levels, the dorsal border of the hippocampus was formed either by the amygdala (medially) or by the lateral ventricle (laterally). The lateral border is formed by the ventricle and the ventral border is the white matter deep to the hippocampus and subiculum. At these rostral levels, the portion of the uncus that connects the caudal amygdala with the hippocampus was included within our measurements. Caudal to the amygdala, the boundaries of the hippocampus are made by the ventricle (dorsally), the laterally adjacent white matter and the white matter subjacent to the subiculum that separates it from the entorhinal cortex (rostrally) and then from the parahippocampal cortex (caudally). The fimbria was not included in the measurements. The posterior boundary of the hippocampus was taken as the section in which the fornix has a dorsomedial trajectory towards the corpus callosum and is distinct from the hippocampal formation. In order to divide the hippocampus into rostral (anterior) and caudal (posterior) portions, the boundary was set at the rostral limit of the lateral geniculate nucleus.

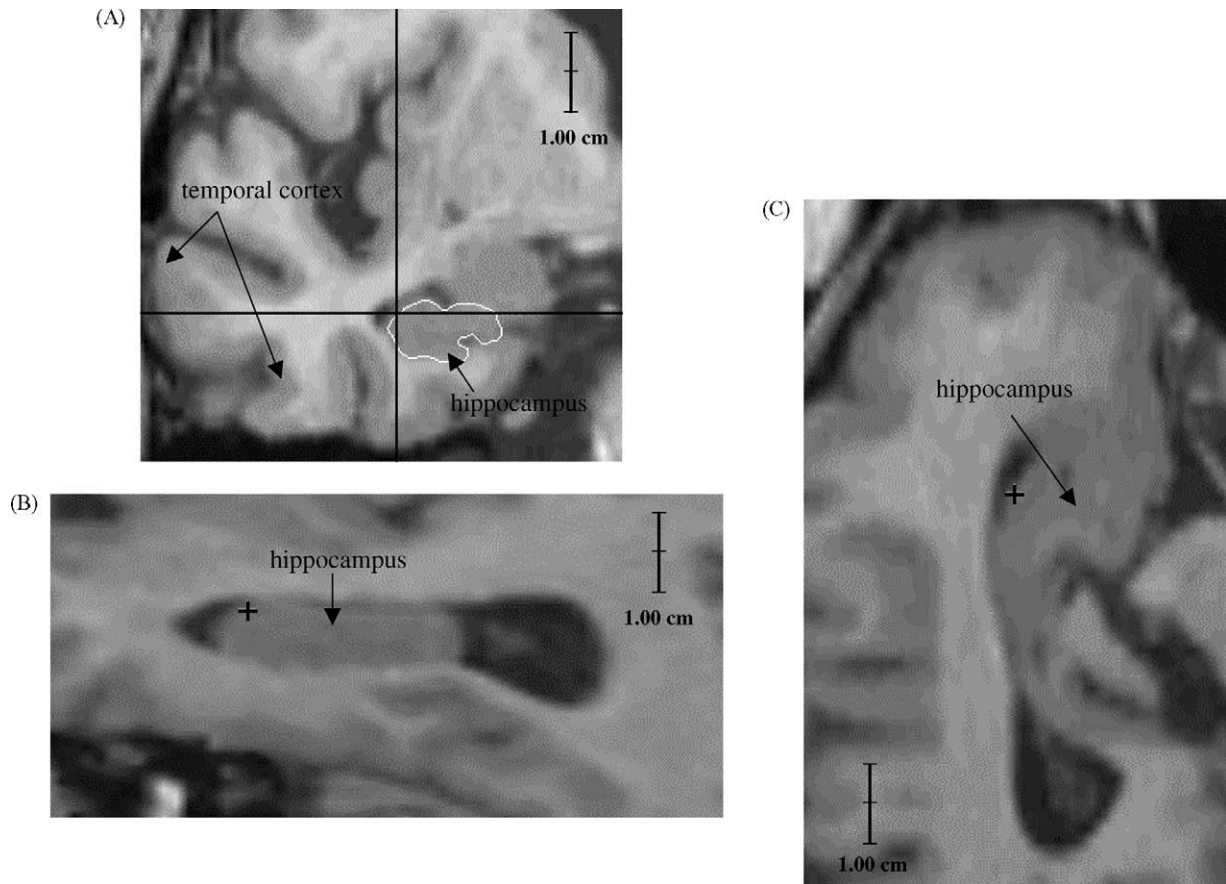


Fig. 1. (A) Outline of the right hippocampus drawn on coronal slice. Viewing the location of the same section of the hippocampus on sagittal (B) and axial (C) slices, as indicated by crosses, helped to guide region drawing.

In order to correct for differences in head size, hippocampal volumes were divided by intracranial volume for each subject. Intracranial volume was determined by manually outlining the margin of the inner table of the skull on contiguous 10 mm axial slices.

2.2. Statistics

Gender differences were evaluated using the Student's *t*-test. Planned comparisons were performed to evaluate the differences between the ERT+ group and the other two groups. Associations between continuous variables were evaluated by regression analysis. Categorical data were evaluated using chi square.

3. Results

Subject characteristics are shown in Table 1. Ten of the women taking estrogen were taking Premarin (0.3–0.625 mg), one was taking Premarin methyltestosterone (0.625 mg), and two were taking prempo (0.625/2.5 mg). Men and women did not differ significantly on age or education, but the men scored higher on the 3MS. When compared to women taking estrogen and women not taking estrogen, men scored higher than the ERT– group on 3MS, but there were no differences between the three groups in age or education. There were no differences between groups in the proportions of cognitively impaired and normal subjects. The two groups of women did not differ with respect

Table 1
Subject characteristics

	Age	Education	3MS	Cognitively impaired (%)
ERT+ (<i>n</i> = 13)	67.00 (60–77) (5.06)	8.15 (4.93)	83.53 (9.58)	15
ERT– (<i>n</i> = 46)	69.43 (60–83) (6.59)	7.73 (5.18)	82.43 ^a (11.60)	19
Men (<i>n</i> = 38)	69.18 (60–83) (5.63)	10.03 (5.30)	87.61 (11.64)	8

Values are means and standard deviations.

^a Less than men (*P* = 0.04).

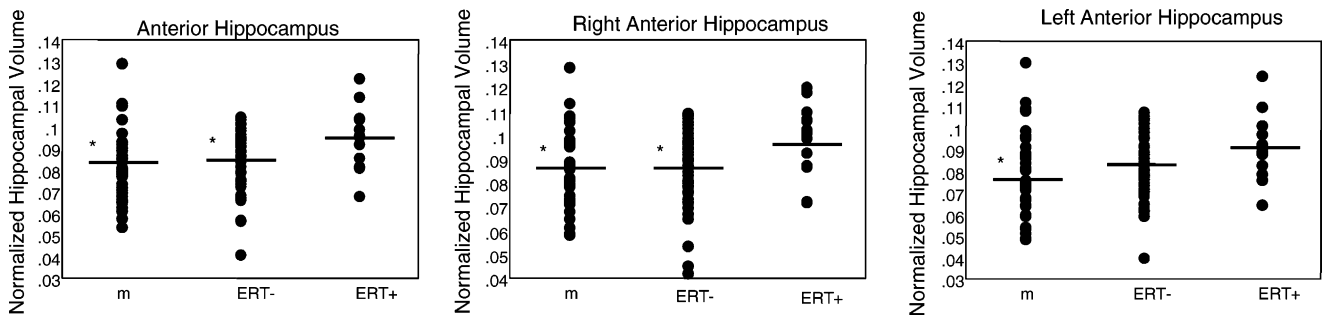


Fig. 2. Scatterplots for each group for the anterior hippocampal volume (averaged over right and left hemispheres) and right and left anterior hippocampal volumes. All volumes are ratios of hippocampal size to intracranial volume. The lines indicate the mean for each group. M, men subjects; ERT–, women subjects not taking estrogen replacement therapy; ERT+, women subjects taking estrogen replacement therapy; (*), less than ERT+, $P < 0.05$.

to hysterectomy status or whether or not they had access to medical care. We were able to obtain information about the onset of menopause, duration of menopause, and onset of menarche for 29 of the women in the ERT– group and 10 of the women in the ERT+ group. The groups did not differ in duration of menopause ($t = 0.103$, $P = 0.92$), onset of menopause ($t = 0.358$, $P = 0.72$), onset of menarche

($t = 0.05$, $P = 0.97$), or the time between menarche and onset of menopause ($t = 0.604$, $\rho = 0.55$).

Hippocampal and intracranial volumes were both drawn with high intra-rater and inter-rater reliability ($\rho = 0.96$ – 0.97). There were no significant effects of gender on normalized hippocampal volumes. Paired comparisons showed that the ERT+ group had significantly larger right

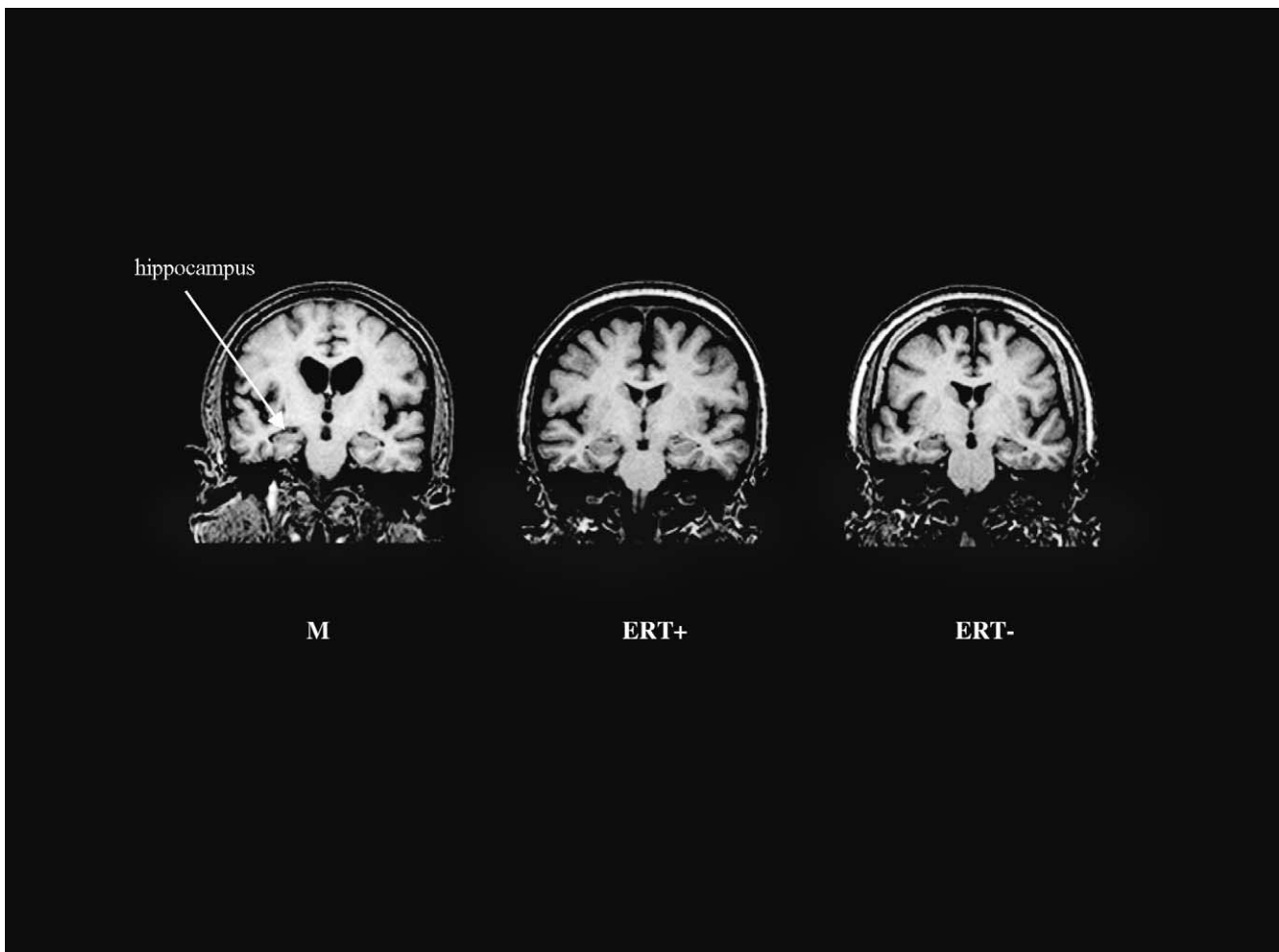


Fig. 3. MRI scans showing the hippocampus for a male subject (M), a woman taking estrogen (ERT+) and a woman not taking estrogen (ERT–). These images demonstrate the average size of the hippocampus for each group. Each subject shown is 70 years of age.

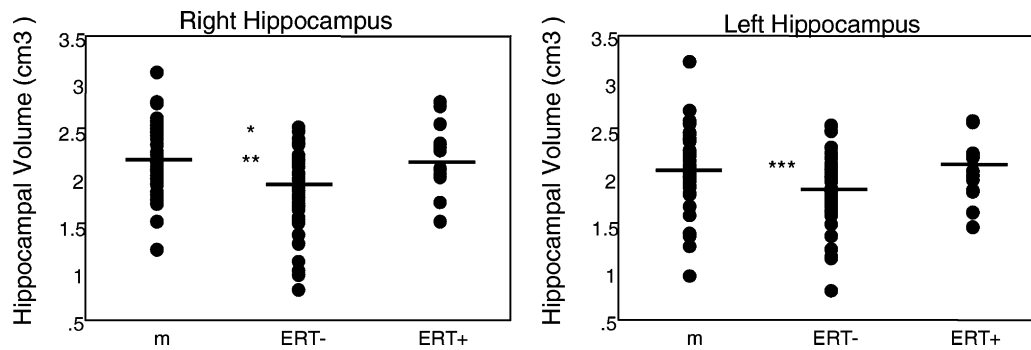


Fig. 4. Scatterplots for each group showing absolute hippocampal volumes (cm^3). The lines indicate the mean for each group. M, men subjects; ERT–, women subjects not taking estrogen replacement therapy; ERT+, women subjects taking estrogen replacement therapy; (*), less than ERT+, $P = 0.01$; (**), less than M, $P < 0.0005$; (***), less than ERT+ and M, $P < 0.05$.

hippocampal volumes than the ERT– group ($t = 2.12$, $P = 0.04$). No further differences were found for whole hippocampal volumes. When divided into the anterior and posterior hippocampus, we found that the ERT+ group had larger anterior hippocampal volumes (averaged over right and left hemispheres) than the ERT– group ($t = 2.29$, $P = 0.03$) and the men ($t = 2.50$, $P = 0.02$). Differences were greater for the right anterior hippocampus between the ERT+ group and the ERT– group ($t = 2.33$, $P = 0.02$) and the men ($t = 2.32$, $P = 0.02$), and were significantly different from the men ($t = 2.20$, $P = 0.03$) but not the ERT– group in the left hemisphere. These data are shown in Fig. 2 and representative images are shown in Fig. 3. No significant differences were found in the posterior hippocampus. For reference to other studies, we also looked at raw hippocampal volumes as shown in Fig. 4. The ERT+ group had significantly larger right and left hippocampal volumes than the ERT– group ($t = 2.60$, $P = 0.01$ and $t = 2.25$, $P = 0.03$, respectively). The men also had larger right and left hippocampal volumes than the ERT– group ($t = 3.76$, $P = 0.0003$ and $t = 2.47$, $P = 0.02$, respectively). The ERT+ group did not differ from the men for either the right or left hippocampus.

Age was significantly associated with right hippocampal volume for the ERT– group ($r = 0.48$, $P = 0.0007$) but not for the men or the ERT+ groups. Age was significantly associated with left hippocampal volume for the ERT– ($r = 0.49$, $P = 0.0006$) and ERT+ ($r = 0.59$, $P = 0.03$) groups but not for the men. The 3MS was significantly associated with right hippocampal volume for the ERT– ($r = 0.48$, $P = 0.0008$) group but not for the other two groups. The 3MS was not associated with left hippocampal volume for any of the groups.

4. Discussion

A growing body of evidence supports a beneficial role of estrogen on cognitive function (see Yaffe et al. [63] for review) and a few neuroimaging studies have shown effects

of estrogen on brain function [13,45,49]. Here, we present evidence that estrogen also has effects on brain structure. Women taking ERT had greater normalized right hippocampal volumes and greater raw hippocampal volumes (right and left) than the women not taking ERT. In addition, women taking ERT had greater anterior hippocampal volumes than both women not taking ERT and men. To our knowledge, this is the first report of an association between estrogen use and hippocampal atrophy.

Gender differences in brain atrophy have been reported in both young and elderly subjects [8,20], although the effects of estrogen have not been well studied. While it has been reported that hippocampal volume is negatively correlated with age in healthy elderly subjects [26,33], it is not clear if there are age-related gender differences in this age group. Only a few studies have looked at gender differences in medial temporal regions with mixed findings. One study found greater age-related hippocampal atrophy in women than in men across a broad age range [40]. However, women between the ages of 20 and 35 years had larger hippocampi than men of the same age and hippocampal volumes were similar for men and women between the ages of 60 and 85 years. Age-related volume declines were not evaluated in young and elderly subjects independently to determine if there were gender differences in age-related atrophy for different age groups that might be associated with hormonal changes. Another study found an age-related decline in temporal lobe gray matter volumes (excluding the hippocampus) but no age-related decline in hippocampal volumes in men ranging from 21 to 70 years of age [54]. Jack et al. [26] reported an age-related decline in medial temporal lobe structures, including the hippocampus, in both elderly men and women. We report an association between hippocampal volume and age for the women but not for the men. The women not taking ERT showed this association in both hemispheres, while the women taking ERT showed a stronger association in the right hemisphere, the same hemisphere in which the greatest volume difference between the two groups of women was observed. The interpretation of these findings is greatly limited, however,

by the small sample size for the women taking ERT. A recent report showed a decline in hippocampal volume in men between the third and fifth decade of life, but relatively constant hippocampal volumes in women of the same age [44]. The authors suggest that the gender differences in younger subjects may reflect the hormonal differences between men and women that are most pronounced in the age group studied. Although the association between estrogen and hippocampal volume was not specifically evaluated in this study, the implication is that estrogen may exert a protective effect that is lost after menopause but that might be sustained by ERT. Our findings provide support for this hypothesis.

There are many possible mechanisms through which estrogen may exert a neuroprotective effect, including modulation of neurotransmitter activity, synaptic reorganization, changes in apolipoprotein E (Apo E) levels, prevention of cerebral ischemia, regulation of amyloid beta precursor protein metabolism, modulation of intracellular calcium, and modulation of growth factor activity [4,24,30,38,53]. A number of rodent studies that have specifically looked at the effects of estrogen on the hippocampus have shown evidence of neuroprotective and neurotrophic effects. For example, estrogen increases the density of dendritic spines and synapses in the CA1 region of the hippocampus [18,61] and regulates the cyclic breakdown of excitatory synapses on dendritic spines [60]. In addition, ovariectomy results in a loss of dendritic spine and synapse density in hippocampal pyramidal cells that is prevented or reversed by estradiol treatment [19], and cell proliferation in the hippocampus is highest when estrogen levels are highest [55]. Fairly recently, estrogen receptor mRNA (ER α and ER β) and ¹²⁵I-estrogen binding have been localized in the pyramidal cells of Ammon's horn providing a mechanism whereby estrogen might modulate hippocampal structure and function via nuclear receptor-mediated events [50]. Given the plethora of possible mechanisms it seems unlikely that estrogen neuroprotection is mediated by a single neuroprotective pathway. Estrogen may exert neuroprotective effects via different mechanisms depending upon neuronal type, type of receptors expressed, extracellular environment, or other factors [19].

It is not clear why the greatest differences were observed in the anterior hippocampus but others have reported similar findings in AD and aging. Jack et al. [26] found that MRI measures of age-related hippocampal volume loss were greatest in the head of the hippocampus in comparison to the body or tail. In addition, AD patients showed the greatest volume differences in the hippocampal head when compared to control subjects. Thus, there is evidence suggesting that the hippocampal head is most susceptible to both age-related and AD-related degeneration and this could explain the current findings.

There are some important shortcomings of this study that warrant discussion. Firstly, we were unable to obtain information about the duration of ERT and whether the women

currently not taking ERT ever took ERT. The lifetime exposure to estrogen may be important with respect to neuroprotective effects. In addition, we were unable to obtain blood or urine levels of estrogen, although earlier reports have found that endogenous estrogen levels were not consistently associated with cognitive performance or risk of cognitive decline in postmenopausal women [3,62]. Still, there may be interindividual differences that were not accounted for that may have influenced the results of this study. The subjects in this study were elderly Mexican American men and women, many with low education levels. Unlike studies with strict inclusionary and exclusionary criteria, the SALSA study is aimed at determining factors that are significant risks for the development of dementia in a representative sample of community dwelling elderly, and therefore includes subjects with a variety of health conditions that may have effects on imaging measures, including hippocampal volume. While this provides an opportunity to evaluate how imaging measures are influenced by different risk factors for dementia, our findings may not generalize to the population as a whole, though there is no a priori reason to believe that these results will not generalize. Still, there may be distinctive genetic, cultural, and biomedical differences between the sampled Mexican American population and other groups of Americans.

This study included subjects with cognitive impairment, but there were no differences in cognitive performance between the three groups. Similarly, our previous report of differences in cerebral glucose metabolism in association with ERT were not associated with cognitive differences [13]. Thus, differences in imaging measures associated with ERT are not necessarily linked with performance on cognitive tests. This may in part reflect differences in the sensitivities of cognitive tests and imaging measures. Performance on the 3MS was associated with right hippocampal volume for the women not taking ERT but not for the other two groups. This is interesting given the fact that the greatest differences in hippocampal volume were observed in the right hemisphere. Larger, well-controlled, longitudinal studies may determine if hippocampal volume loss is associated with a decline in cognitive function. Several recent studies have shown that ERT does not improve cognitive function in AD patients [22,39,59]. Rather, a variety of findings, including the MRI findings presented here, show a positive effect of ERT on measures associated with dementia, suggesting that ERT may protect against the development or slow the onset of dementia. It was recently reported that the rate of hippocampal volume loss in subjects with MCI was greater in those who subsequently converted to AD than in those who remained clinically stable [25]. Follow-up cognitive testing on the subjects in the current study will determine if they decline or develop dementia, if hippocampal volume is predictive of future decline in these subjects, and if ERT has an influence on the occurrence or rate of future cognitive decline.

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