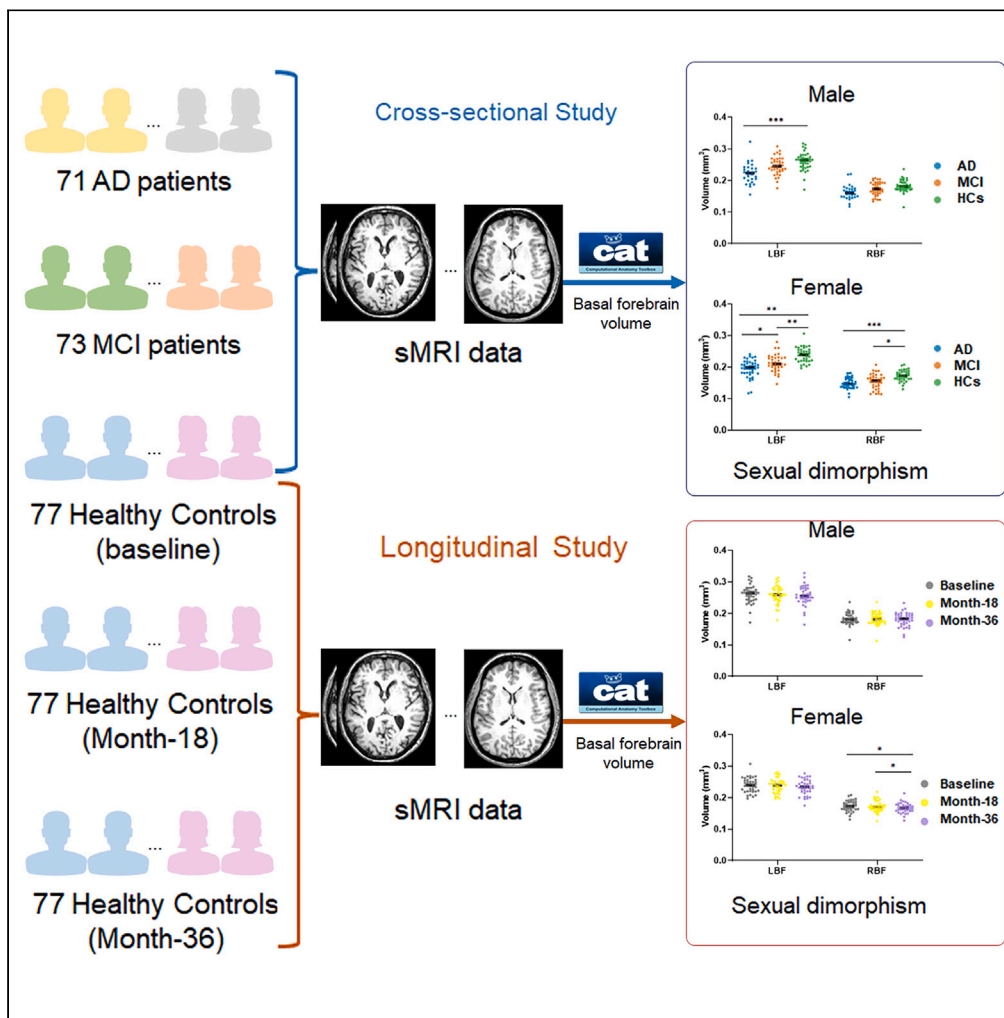## Article

Exploring sexual dimorphism in basal forebrain volume changes during aging and neurodegenerative diseases

Yajun Shi, Dong Cui, Fengzhu Sun, ..., Qing Jiao, Weifang Cao, Guanghui Yu

cuidong_cd@126.com (D.C.)
ghyu@sdfmu.edu.cn (G.Y.)

Highlights

BF volume reduction is an imaging marker for diagnosing neurodegenerative diseases

Asymmetric atrophy was observed in the Ch4 subregion in female patients with AD

Aging appeared to have a minimal effect on BF volume in the normal elderly males

Article

Exploring sexual dimorphism in basal forebrain volume changes during aging and neurodegenerative diseases

Yajun Shi,[1,2,4] Dong Cui,[1,2,4,5,*] Fengzhu Sun,[1,2] Zhen OuYang,[2,3] Ruhai Dou,[1,2] Qing Jiao,[1,2] Weifang Cao,[1,2] and Guanghui Yu[1,2,6,*]

SUMMARY

Patients with neurodegenerative diseases exhibit diminished basal forebrain (BF) volume compared to healthy individuals. However, it's uncertain whether this difference is consistent between sexes. It has been reported that BF volume moderately atrophies during aging, but the effect of sex on BF volume changes during the normal aging process remains unclear. In the cross-sectional study, we observed a significant reduction in BF volume in patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD) compared to Healthy Controls (HCs), especially in the Ch4 subregion. Notably, significant differences in BF volume between MCI and HCs were observed solely in the female group. Additionally, we identified asymmetrical atrophy in the left and right Ch4 subregions in female patients with AD. In the longitudinal analysis, we found that aging seemed to have a minimal impact on BF volume in males. Our study highlights the importance of considering sex as a research variable in brain science.

INTRODUCTION

The basal forebrain cholinergic system (BFCS) is situated in the anterior and ventral regions of the striatum. It consists of four distinct groups of cholinergic cells. Ch1 refers to medial septal nucleus–related cells, Ch2 and Ch3 correspond to those associated with the vertical and horizontal limbs of the diagonal band of Broca, respectively, and Ch4 represents the largest nucleus within the BFCS, comprising cells of the nucleus basalis of Meynert.[1,2] Basal forebrain (BF) neurons provide the major cholinergic innervation to the brain neocortex, hippocampus, amygdala, and some thalamic nuclei.[3] They play an important role in cognitive function and attentional behaviors.[4] Cholinergic deficiency has been well demonstrated to be partly responsible for age–related cognitive deficits and neurodegenerative diseases.[5–8] The loss of cortical cholinergic activity resulting from the neuronal dysfunction of the BFCS is one of the principal features of Alzheimer's disease (AD).[9] General intelligence has been found to be significantly associated with BF volume in healthy elderly people.[2] Further, it has been found that the manipulation of BF cholinergic neurons in mammalian development may bring about different behavioral effects in the two sexes[3]; however, the sexual dimorphism in BF volume differences between patients with neurodegenerative diseases and healthy controls (HCs), as well as the sexual dimorphism in BF volume changes during the normal aging process, remains unclear.

In recent years, progress in non–invasive, high–resolution magnetic resonance imaging (MRI) technology, along with the refinement of brain atlases, has facilitated the precise *in vivo* measurement of BF morphological parameters during aging and neurodegenerative diseases. Research combining structural MRI (sMRI) and cerebrospinal fluid (CSF) examination has revealed that cognitively normal older adults with abnormal amyloid beta and tau pathology in CSF biomarkers exhibit a reduction in gray matter (GM) volume within the Ch4 subregion of the BF.[10] A longitudinal sMRI study found that in cognitively normal participants destined to develop AD, the BF area exhibited significant atrophy as early as 4.5 years before the onset of clinical symptoms, indicating that atrophy in the BF is a biomarker that predicts the likelihood of asymptomatic elderly subjects developing AD.[11] Furthermore, a signal decrease in proton density MRI in patients with AD in the BF may be related to the loss or degeneration of cholinergic neurons and may correspond to regional cortical GM atrophy.[12] It has been found that the BFCS is atrophied in patients with mild cognitive impairment (MCI),[13] and the volume loss of BF is associated with cognitive decline in patients with MCI.[14]

It has been reported that approximately two–thirds of patients with AD are females,[15] and they tend to experience faster cognitive decline compared to males.[16] This could be due to the brain of female patients with AD undergo more severe pathological damage, leading to more pronounced hippocampal atrophy and cognitive decline.[17] Extensive brain imaging studies have provided evidence that males with MCI and

[1]Department of Radiology, The Second Affiliated Hospital of Shandong First Medical University, Tai' an, Shandong 271000, China
[2]School of Radiology, Shandong First Medical University and Shandong Academy of Medical Sciences, Tai' an, Shandong 271016, China
[3]Department of Radiology, Taian Municipal Hospital, Tai' an, Shandong 271000, China
[4]These authors contributed equally
[5]Senior author
[6]Lead contact
*Correspondence: cuidong_cd@126.com (D.C.), ghyu@sdfmu.edu.cn (G.Y.)

Table 1. The comparisons of BF subregion volumes among the three groups

Characteristics	AD (n = 71)	MCI (n = 73)	HCs (n = 77)	F/χ^2 value	p value	F value interaction	p value interaction	Post–hoc AD vs. MCI	AD vs. HCs	MCI vs. HCs
Age	73.70 ± 8.079	74.27 ± 6.321	71.78 ± 5.924	2.710[b]	0.069	0.795	0.453	1.000	0.343	0.075
Sex (male/female)	28/43	37/36	40/37	2.759[a]	0.252	–	–	–	–	–
MMSE	20.41 ± 5.515	27.27 ± 1.895	28.96 ± 1.175	121.953[b]	<0.001	1.682	0.189	<0.001	<0.001	0.007
LBF	0.206 ± 0.032	0.229 ± 0.032	0.250 ± 0.028	29.135[c]	*<0.001*	2.526	0.358	*<0.001*	*<0.001*	*0.012*
RBF	0.155 ± 0.020	0.164 ± 0.024	0.178 ± 0.019	10.469[c]	*<0.001*	2.086	0.358	0.293	*<0.001*	0.081
BF Asymmetry	0.282 ± 0.123	0.331 ± 0.111	0.338 ± 0.095	3.410[c]	*0.045*	0.647	0.596	0.097	0.097	1.000
LBF1–3	0.096 ± 0.012	0.099 ± 0.015	0.105 ± 0.014	3.399[c]	*0.045*	0.637	0.596	1.000	0.120	0.143
RBF1–3	0.058 ± 0.011	0.059 ± 0.013	0.064 ± 0.012	0.895[c]	0.461	1.281	0.420	1.000	1.000	1.000
BF1–3 Asymmetry	0.490 ± 0.173	0.509 ± 0.223	0.493 ± 0.183	0.079[c]	0.924	0.504	0.605	1.000	1.000	1.000
LBF4	0.110 ± 0.024	0.131 ± 0.022	0.145 ± 0.019	36.972[c]	*<0.001*	3.124	0.358	*<0.001*	*<0.001*	*0.022*
RBF4	0.096 ± 0.014	0.105 ± 0.016	0.114 ± 0.012	18.438[c]	*<0.001*	1.529	0.394	*0.019*	*<0.001*	*0.034*
BF4 Asymmetry	0.121 ± 0.198	0.213 ± 0.118	0.238 ± 0.115	9.220[c]	*<0.001*	1.852	0.358	*0.003*	*<0.001*	1.000

Data was presented as mean ± standard deviation.

n, the number of subjects; AD, Alzheimer's disease; MCI, mild cognitive impairment; HCs, healthy controls; MMSE, Mini–Mental State Examination; LBF, left basal forebrain; RBF, right basal forebrain; BF Asymmetry, volume difference between the left and right basal forebrain; LBF1–3, left basal forebrain Ch1–3; RBF1–3, right basal forebrain Ch1–3; BF1–3 Asymmetry, volume difference between the left and right basal forebrain Ch1–3; LBF4, left basal forebrain Ch4; RBF4, right basal forebrain Ch4; BF4 Asymmetry, volume difference between the left and right basal forebrain Ch4.

[a]Pearson chi–square test.

[b]One–way ANOVA.

[c]One–way ANCOVA. p values corrected by False Discovery Rate (FDR) correction were considered statistically significant and represented as bold and slant bodies.

AD tend to experience slower rates of brain atrophy over time compared to females.[18] In addition, it has been observed that males with MCI show less atrophy in multiple brain regions, and once diagnosed with AD, they exhibit less atrophy in various regions as well.[19,20] It has been proposed that cholinergic basal forebrain cortical projection neurons within the nucleus basalis, which mediate memory, attention, and the degeneration in AD, may exhibit greater vulnerability in elderly females compared to males.[21] Studies have also indicated that sex hormones have an impact on cholinergic basal forebrain functioning.[3,22,23] Animal experiments have further revealed significant sex differences in estrogen receptors within BF cholinergic neurons.[24] A recent study found that sex impacted the associations between BF dynamics and global fibrillary amyloid–β pathology in cognitively normal older adults with subjective memory complaints.[25]

The purpose of this study is to investigate the sexual dimorphism in BF volume differences between patients with neurodegenerative diseases and HCs, and the sexual dimorphism in BF volume changes during the normal aging process. First, we aim to conduct a cross–sectional comparison of BF volume differences among three groups—AD, MCI, and HCs—and analyze the sexual dimorphism in BF volume differences between patients with neurodegenerative diseases and HCs. In addition, we intend to longitudinally investigate the changes in BF volume over time in cognitively normal older adults and analyze the influence of sex.

RESULTS

Cross–sectional study

The demographic information and brain region volumes of the three groups are presented in Table 1. There were no significant differences in age (F = 2.710; p = 0.069) or sex (χ^2 = 2.759; p = 0.252) among the three groups. MMSE scores (F = 121.953; p < 0.001) showed significant
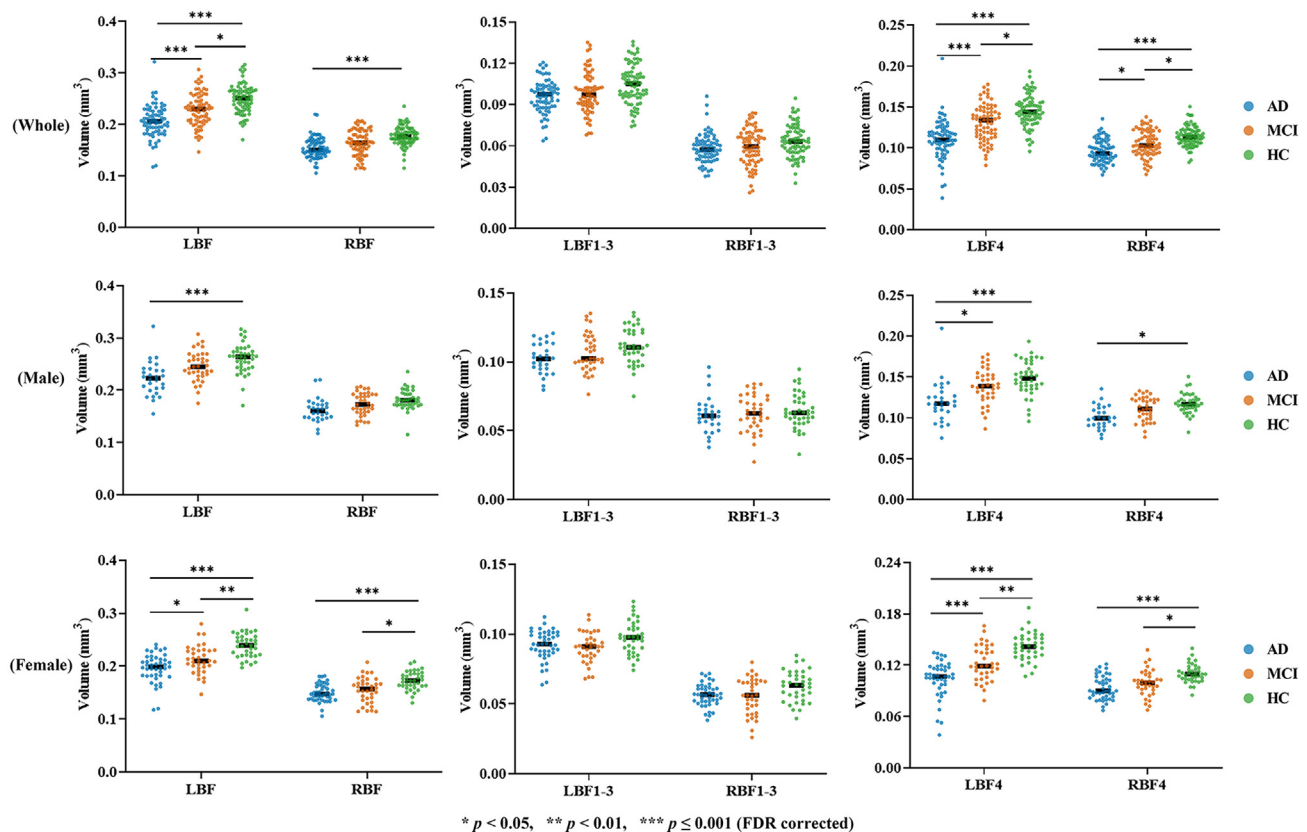
Figure 1. The volumes of the BF subregion of three groups
There were significant differences in the total volume of LBF among the three groups, while significant differences in the RBF were observed solely between the AD and HCs groups. The significant differences in BF4 volume were observed bilaterally among three groups. However, there were no differences in the volume of Ch1–3 among the three groups. Furthermore, significant differences in BF volumes between the MCI and HCs groups were exclusively observed in females, indicating that the atrophy of the BF can serve as a reliable imaging marker for the early diagnosis of MCI specifically in females. ($*p < 0.05$, $**p < 0.01$, $***p \leq 0.001$, one–way ANCOVA, FDR corrected).

differences among the three groups. After the FDR correction, as shown in Table 1, there were notable differences in the volume of BF among the three groups, thus suggesting that the BF volumes of AD and MCI subjects decreased significantly compared with HCs, particularly in the LBF, while significant differences in the RBF were observed solely between the AD and HC groups. The decrease in BF volume was primarily attributed to the atrophy of Ch4, as noteworthy statistical differences were observed in the bilateral BF4 among the three groups; however, there were no differences in the volumes of Ch1–3 among the three groups (Figure 1, first row). These findings suggested that BF volume, particularly the Ch4 subregion volume, can serve as an imaging marker in the diagnosis of neurodegenerative diseases.

As shown in Table 1, there was no significant interaction between group and sex. As a result, we separately investigated the differences in BF volumes among the three groups for males and females, aiming to explore the sexual dimorphism in differences between groups. As shown in Tables 2 and 3, there was no significant difference in Ch4 subregion volume (p = 1.000, FDR corrected) between MCI and HCs in males (Figure 1, second row). In contrast, we observed significant differences in the Ch4 subregion volume among all three groups in females (Figure 1, third row). These results suggested that the Ch4 subregion volume can serve as a reliable imaging marker for the early diagnosis of MCI, particularly in females.

Interestingly, we found a significant difference in BF4 asymmetry between the AD and HC groups as well as between the AD and MCI groups; however, these significant differences were exclusively observed in females. Specifically, BF4 asymmetry was significantly reduced in the AD group compared to the HC and MCI groups (Figure 2).

Longitudinal study

According to the findings presented in Table 4, there were no significant differences in MMSE scores across the three follow–up assessments, indicating that participants remained cognitively normal throughout each follow–up period. Following the FDR correction, there were no significant differences in the volume of LBF, RBF, BF asymmetry, LBF1–3, BF1–3 asymmetry, RBF4, or BF4 asymmetry. However, we observed a significant reduction in the volume of RBF1–3 and LBF4 at month 36 compared to the baseline (Figure 3, first row).

Table 2. The comparisons of BF subregion volumes in males among the three groups

Characteristics	AD (n = 28)	MCI (n = 37)	HCs (n = 40)	F/χ^2 value	p value	Post–hoc		
						AD vs. MCI	AD vs. HCs	MCI vs. HCs
Age	72.86 ± 8.013	74.59 ± 5.188	72.45 ± 5.875	1.214[a]	0.301	0.820	1.000	0.416
MMSE	21.43 ± 5.181	27.24 ± 1.754	28.87 ± 1.343	52.688[a]	<0.001	<0.001	<0.001	0.056
LBF	0.222 ± 0.033	0.246 ± 0.028	0.260 ± 0.028	8.284[b]	*<0.001*	0.059	*<0.001*	1.000
RBF	0.162 ± 0.023	0.174 ± 0.021	0.182 ± 0.019	2.276[b]	0.214	0.918	0.467	1.000
BF Asymmetry	0.310 ± 0.124	0.343 ± 0.105	0.353 ± 0.086	0.667[b]	0.663	1.000	1.000	1.000
LBF1–3	0.102 ± 0.011	0.107 ± 0.014	0.112 ± 0.013	1.095[b]	0.509	1.000	1.000	1.000
RBF1–3	0.062 ± 0.013	0.063 ± 0.013	0.065 ± 0.012	0.001[b]	1.000	1.000	1.000	1.000
BF1–3 Asymmetry	0.505 ± 0.188	0.526 ± 0.225	0.541 ± 0.163	0.119[b]	0.999	1.000	1.000	1.000
LBF4	0.120 ± 0.025	0.139 ± 0.021	0.149 ± 0.021	9.693[b]	*<0.001*	*0.027*	*<0.001*	1.000
RBF4	0.101 ± 0.014	0.111 ± 0.015	0.118 ± 0.012	5.652[b]	*0.015*	0.248	*0.027*	1.000
BF4 Asymmetry	0.166 ± 0.162	0.221 ± 0.115	0.229 ± 0.119	2.177[b]	0.214	0.557	0.750	1.000

Data was presented as mean ± standard deviation.

n, the number of subjects; AD, Alzheimer's disease; MCI, mild cognitive impairment; HCs, healthy controls; MMSE, Mini–Mental State Examination; LBF, left basal forebrain; RBF, right basal forebrain; BF Asymmetry, volume difference between the left and right basal forebrain; LBF1–3, left basal forebrain Ch1–3; RBF1–3, right basal forebrain Ch1–3; BF1–3 Asymmetry, volume difference between the left and right basal forebrain Ch1–3; LBF4, left basal forebrain Ch4; RBF4, right basal forebrain Ch4; BF4 Asymmetry, volume difference between the left and right basal forebrain Ch4.

[a]One–way ANOVA.

[b]One–way ANCOVA. p values corrected by False Discovery Rate (FDR) correction were considered statistically significant and represented as bold and slant bodies.

No significant interaction was found between time and sex, as indicated in Table 4. Consequently, we proceeded to analyze the differences separately for males and females at the three follow–up visits. As shown in Table 5, no significant differences were observed in the volumes of the BF subregion or in asymmetry in males across the three follow–up visits, indicating that aging had a minimal effect on BF volume in healthy older males (Figure 3, second row). However, in females, as presented in Table 6, there was a significant decrease in the volume of RBF

Table 3. The comparisons of BF subregion volumes in females among the three groups

Characteristics	AD (n = 43)	MCI (n = 36)	HCs (n = 37)	F/χ^2 value	p value	Post–hoc		
						AD vs. MCI	AD vs. HCs	MCI vs. HCs
Age	74.26 ± 8.168	73.94 ± 7.368	71.05 ± 5.972	2.245[a]	0.111	1.000	0.157	0.278
MMSE	19.74 ± 5.682	27.31 ± 2.054	29.05 ± 0.970	69.237[a]	<0.001	<0.001	<0.001	0.202
LBF	0.196 ± 0.027	0.213 ± 0.027	0.239 ± 0.023	23.806[b]	*<0.001*	*0.018*	*<0.001*	*0.004*
RBF	0.150 ± 0.017	0.155 ± 0.024	0.173 ± 0.017	10.328[b]	*<0.001*	0.927	*<0.001*	*0.018*
BF Asymmetry	0.263 ± 0.120	0.318 ± 0.117	0.322 ± 0.103	3.279[b]	0.062	0.166	0.181	1.000
LBF1–3	0.092 ± 0.011	0.090 ± 0.011	0.097 ± 0.012	2.695[b]	0.093	1.000	0.354	0.174
RBF1–3	0.056 ± 0.008	0.056 ± 0.013	0.063 ± 0.011	2.144[b]	0.137	1.000	0.431	0.273
BF1–3 Asymmetry	0.491 ± 0.164	0.491 ± 0.223	0.440 ± 0.191	0.407[b]	0.667	1.000	1.000	1.000
LBF4	0.104 ± 0.021	0.122 ± 0.020	0.142 ± 0.016	32.020[b]	*<0.001*	*<0.001*	*<0.001*	*0.007*
RBF4	0.093 ± 0.014	0.099 ± 0.015	0.110 ± 0.011	14.381[b]	*<0.001*	0.181	*<0.001*	*0.018*
BF4 Asymmetry	0.092 ± 0.216	0.206 ± 0.122	0.247 ± 0.112	8.164[b]	*<0.001*	*0.018*	*0.004*	1.000

Data was presented as mean ± standard deviation.

n, the number of subjects; AD, Alzheimer's disease; MCI, mild cognitive impairment; HCs, healthy controls; MMSE, Mini–Mental State Examination; LBF, left basal forebrain; RBF, right basal forebrain; BF Asymmetry, volume difference between the left and right basal forebrain; LBF1–3, left basal forebrain Ch1–3; RBF1–3, right basal forebrain Ch1–3; BF1–3 Asymmetry, volume difference between the left and right basal forebrain Ch1–3; LBF4, left basal forebrain Ch4; RBF4, right basal forebrain Ch4; BF4 Asymmetry, volume difference between the left and right basal forebrain Ch4.

[a]One–way ANOVA.

[b]One–way ANCOVA. p values corrected by False Discovery Rate (FDR) correction were considered statistically significant and represented as bold and slant bodies.
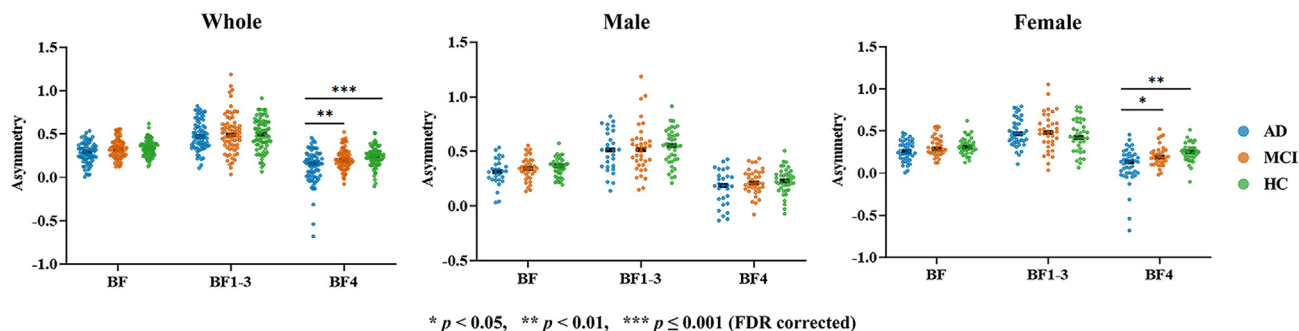
Figure 2. The left–right asymmetry of the BF subregion of three groups

There was a significant difference in BF4 asymmetry between the AD and HCs groups and between the AD and MCI groups. Specifically, BF4 asymmetry was significantly reduced in the AD group compared to the HCs and MCI groups. Notably, this asymmetrical difference was found exclusively in females, implying that female patients with AD exhibit asymmetric atrophy in the Ch4 region. There was no significant difference in left–right asymmetry among the three groups in males. (*p < 0.05, **p < 0.01, ***p ≤ 0.001, one–way ANCOVA, FDR corrected).

and LBF4 during the normal aging process (Figure 3, third row). These findings demonstrated the presence of sexual dimorphism in the changes in BF subregion volumes during the normal aging process.

DISCUSSION

This study employed both cross–sectional and longitudinal designs to investigate the impact of neurodegenerative diseases and aging on BF volume and to explore sexual dimorphism. The key findings were as follows: 1) patients with AD exhibited a decrease in BF volume, particularly in the Ch4 subregion, even before the formal diagnosis of AD. 2) There was sexual dimorphism in the differences in BF subregion volumes between individuals with neurodegenerative diseases and HCs. The volume of the Ch4 subregion can serve as a dependable imaging marker for the early diagnosis of MCI only in females. 3) There was asymmetrical atrophy in the left and right Ch4 subregions in female patients with AD. 4) There was sexual dimorphism in the changes in BF subregion volumes during the normal aging process. Aging appeared to have a minimal effect on BF volume in the male general elderly population. These findings provided evidence for the use of BF volume as an imaging marker for the diagnosis of neurodegenerative diseases and emphasized the importance of considering sex as a crucial variable in brain science research.

The aim of our cross–sectional study was to analyze the differences of BF volume between the neurodegenerative diseases and HCs and explore the sexual dimorphism. Our findings indicated a significant reduction in BF volume, specifically in the Ch4 region, even before the diagnosis of AD. Most of the cholinergic innervation of the cortex, which is involved in attention and memory, originates in the Ch4 (nucleus basalis of Meynert) and in the horizontal limb of the diagonal band nucleus of the basal prosencephalon. Functional alterations in this system have been implicated in neurocognitive disorders as well as the cognitive changes described in Parkinson's disease and AD.[26] The atrophy of BF in patients with AD and MCI has been widely reported.[27–29] A study utilizing a large multicenter dataset found that all of the subregion volumes of the BFCS were significantly reduced in the AD group.[30] Among these subregions, the volume reduction in the nucleus basalis of Meynert (NbM) was particularly pronounced.[27,30] Studies based on sMRI have indicated that a decrease in hippocampal and BF volume is associated with an increased risk of MCI progressing to AD.[29] A recent multimodal MRI study revealed that, compared to the normal control group, the cholinergic BF volumes and mean diffusivity were significantly different in patients with MCI and AD but not in individuals with subjective cognitive decline.[31] Combined with our findings, these findings suggest that the morphological parameters of the BF, particularly Ch4, can serve as robust, reliable imaging markers for the diagnosis of neurodegenerative diseases.

There was sexual dimorphism in the differences in BF subregion volumes between individuals with neurodegenerative diseases and HCs. The volume of the Ch4 subregion can serve as a reliable imaging marker for the early diagnosis of MCI only in females. In the MCI stage, significant decline in BF volume was observed in females, while in males, significant atrophy was observed in the AD stage. The incidence of AD is often reported to be higher for females than for males,[15] which indicates that sex plays an important role in AD brain research. Evidence has suggested that the cholinergic system develops in a sexually dimorphic manner.[3,32] The survival and maintenance of BFCS neurons rely on the nerve growth factor (NGF) and its homologous receptors (trkA and p75[NTR]).[21] Compared to HC, p75(NTR) mRNA levels in BF cholinergic neurons decreased by approximately 40% in females with AD, while the p75(NTR) expression in males remained unchanged. Additionally, compared to HC and MCI individuals, males with AD demonstrated a 45% decrease in trkA mRNA levels in BF cholinergic neurons, while females exhibited a 50% decrease, and reduced trkA mRNA levels were associated with poorer global cognitive performance in females. These findings suggested that females may be at a higher risk of cholinergic BF neuron degeneration.[21] Similar findings have been obtained in experiments involving ovariectomy in rats.[33] Furthermore, Cantero demonstrated for the first time that atrophy of the NbM in the MCI stage is associated with structural changes in the cerebral cortex in humans, and this relationship is more pronounced in females.[34] These findings emphasized the importance of investigating the mechanisms of neurodegenerative diseases separately for males and females.

Table 4. The comparisons of BF subregion volumes of three follow–up visits

Characteristics	Baseline (n = 77)	Month–18 (n = 77)	Month–36 (n = 77)	F value interaction	p value interaction	F value	p value	Baseline vs. Month–18	Baseline vs. Month–36	Month–18 vs. Month–36
Age range	61–86	–	–	–	–	–	–	–	–	–
Sex	Males (n = 40), Females (n = 37)			–	–	–	–	–	–	–
MMSE	28.96 ± 1.175	28.86 ± 1.189	28.90 ± 1.107	0.262	0.770	0.262	0.770	1.000	1.000	1.000
LBF	0.250 ± 0.028	0.249 ± 0.027	0.245 ± 0.030	0.217	0.806	3.309	0.074	1.000	0.176	0.611
RBF	0.178 ± 0.019	0.178 ± 0.019	0.174 ± 0.020	1.335	0.269	3.772	0.065	1.000	0.173	0.220
BF Asymmetry	0.338 ± 0.095	0.334 ± 0.088	0.337 ± 0.091	0.178	0.837	0.120	0.887	1.000	1.000	1.000
LBF1–3	0.105 ± 0.014	0.106 ± 0.015	0.105 ± 0.014	0.111	0.895	0.354	0.791	1.000	1.000	1.000
RBF1–3	0.064 ± 0.012	0.063 ± 0.011	0.061 ± 0.011	0.049	0.952	7.319	*0.009*	1.000	*0.041*	0.153
BF1–3 Asymmetry	0.493 ± 0.183	0.507 ± 0.188	0.530 ± 0.186	0.085	0.918	3.715	0.065	1.000	0.169	0.611
LBF4	0.145 ± 0.019	0.143 ± 0.018	0.140 ± 0.019	0.785	0.459	6.046	*0.014*	1.000	*0.041*	0.537
RBF4	0.114 ± 0.012	0.115 ± 0.013	0.113 ± 0.014	2.311	0.106	0.844	0.558	1.000	1.000	1.000
BF4 Asymmetry	0.228 ± 0.115	0.220 ± 0.102	0.211 ± 0.108	0.236	0.790	1.730	0.275	1.000	0.611	1.000

Data was presented as mean ± standard deviation. All p values were calculated by using linear mixed effect model. p values corrected by False Discovery Rate (FDR) correction were considered statistically significant and represented as bold and slant bodies.

n, the number of subjects; MMSE, Mini–Mental State Examination; LBF, left basal forebrain; RBF, right basal forebrain; BF Asymmetry, volume difference between the left and right basal forebrain; LBF1–3, left basal forebrain Ch1–3; RBF1–3, right basal forebrain Ch1–3; BF1–3 Asymmetry, volume difference between the left and right basal forebrain Ch1–3; LBF4, left basal forebrain Ch4; RBF4, right basal forebrain Ch4; BF4 Asymmetry, volume difference between the left and right basal forebrain Ch4.

In addition, we observed a significant difference in left–right brain asymmetry between female patients with AD and HC individuals, with decreased left–right BF4 asymmetry in patients with AD. The left and right hemispheres of the human brain exhibit slight asymmetry,[35,36] and studies have suggested that there are abnormalities in brain asymmetry in several diseases, such as schizophrenia, depressive disorders, anxiety, and neurodegenerative disease.[37–40] Numerous other factors may also contribute to brain asymmetry, such as genes, sex, and hormones.[40] Many studies have suggested that the brain's left–right asymmetry is one of the possible diagnostic landmarks for AD.[41,42] Clinical research has found that, as patients progress from HC to AD, the size and shape of many anatomical structures in the brain, such as the hippocampus and basal forebrain, undergo changes in the left and right hemispheres, and the asymmetry also changes.[42,43] A recent study that investigated the complexity of the cerebral cortex using fractal dimensions found that the leftward hemispheric and temporal lobe asymmetry decreased with age, and males had significantly lower asymmetry between hemispheres and higher asymmetry in the parietal and occipital lobes than females.[44] Furthermore, research that utilized a resting–state functional connectivity–based gradient approach to assess asymmetries found that males exhibited greater leftward asymmetry than females.[45] It has been demonstrated that the left NbM has higher neuronal density, while the right NbM has higher glial cell density, and the asymmetry in BF structures tends to be sex–dependent, with a tendency toward somewhat greater asymmetry in males.[46] Our findings highlighted the possibility of differing temporal dynamics in Ch4 atrophy between the two hemispheres in females and the importance of considering sex differences in AD–related research.

In the longitudinal study, we found that there was sexual dimorphism in the changes in BF subregion volumes during the normal aging process. Aging appeared to have a minimal effect on BF volume in the male general elderly population. However, there was a significant decrease in the BF volume with increasing age in females. Previous research findings indicated that sex hormones influence the function of cholinergic neurons in animals and humans. There was a higher proportion of neurons showing cytoplasmic androgen receptor expression in females compared to males.[23] An animal experiment showed that estrogen had a protective effect on both male and female BF cholinergic fibers, and the therapeutic potential of estrogen decreased with increasing age.[47] The BFCS has been found to undergo moderate neurodegeneration during normal aging.[27] A recent review demonstrated a correlation between cognitive impairment and the decline in cholinergic cell numbers during aging,[48] and our study indicated that this impairment might be more prominent in females. These observations suggested a potential sexual dimorphism in the changes of BF volume during the normal aging process.
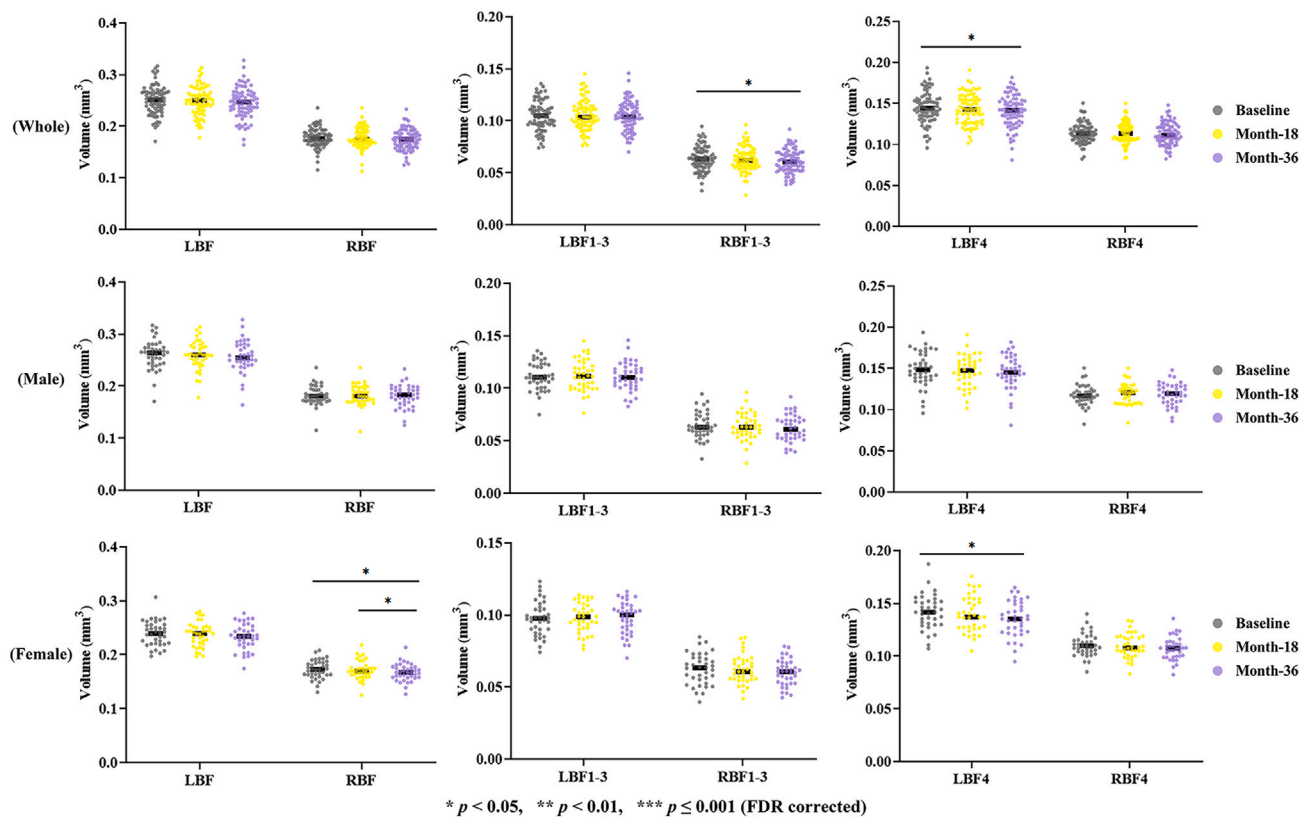
Figure 3. The volumes of the BF subregion of three follow–up visits

No significant differences were observed in the volume of BF subregion in males across the three follow–up visits, indicating that aging had a minimal effect on BF volume in healthy older males. However, there was a significant decrease in the volume of RBF and LBF4 during the normal aging process in females. (*p < 0.05, **p < 0.01, ***p ≤ 0.001, linear mixed effect model, FDR corrected).

Limitations of the study

There are several limitations that should be considered in the present study. First, it should be noted that the diagnosis of MCI and AD was not confirmed through the demonstration of AD biomarkers, which is a more precise strategy. Second, we did not take into account certain electrophysiological parameters of the participants, such as body mass index, blood pressure, blood lipids, and alcohol consumption, which have been shown to impact the volume of brain regions.[49–52] Finally, we did not investigate the relationship between cognitive functions, such as episodic memory and executive function, and the volume of specific subregion of the BF. We intend to include cognitive measures in future research to better understand how changes in BF volume correlate with cognition.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - MRI data acquisition method details
  - Image processing and volume estimation of BF
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Cross–sectional study
  - Longitudinal study

Table 5. The comparisons of BF subregion volumes in males of three follow–up visits

Characteristics	Baseline (n = 40)	Month–18 (n = 40)	Month–36 (n = 40)	F value	p value	Baseline vs. Month–18	Baseline vs. Month–36	Month–18 vs. Month–36
Age range	64–86	–	–	–	–	–	–	–
MMSE	28.88 ± 1.343	28.88 ± 1.159	28.88 ± 1.137	0.001	1.000	1.000	1.000	1.000
LBF	0.260 ± 0.028	0.259 ± 0.028	0.257 ± 0.031	0.853	0.650	1.000	1.000	1.000
RBF	0.182 ± 0.019	0.183 ± 0.020	0.181 ± 0.022	0.455	0.815	1.000	1.000	1.000
BF Asymmetry	0.353 ± 0.086	0.344 ± 0.085	0.346 ± 0.079	0.205	0.815	1.000	1.000	1.000
LBF1–3	0.112 ± 0.013	0.112 ± 0.015	0.111 ± 0.013	0.293	0.815	1.000	1.000	1.000
RBF1–3	0.065 ± 0.012	0.064 ± 0.013	0.062 ± 0.013	3.122	0.486	1.000	1.000	1.000
BF1–3 Asymmetry	0.541 ± 0.163	0.559 ± 0.177	0.577 ± 0.162	1.784	0.574	1.000	1.000	1.000
LBF4	0.149 ± 0.021	0.147 ± 0.019	0.145 ± 0.020	1.346	0.574	1.000	1.000	1.000
RBF4	0.118 ± 0.012	0.119 ± 0.013	0.119 ± 0.014	1.174	0.574	1.000	1.000	1.000
BF4 Asymmetry	0.229 ± 0.119	0.203 ± 0.110	0.197 ± 0.110	1.694	0.574	1.000	1.000	1.000

Data was presented as mean ± standard deviation. All p values were calculated by using linear mixed effect model. p values corrected by False Discovery Rate (FDR) correction were considered statistically significant and represented as bold and slant bodies.
n, the number of subjects; MMSE, Mini–Mental State Examination; LBF, left basal forebrain; RBF, right basal forebrain; BF Asymmetry, volume difference between the left and right basal forebrain; LBF1–3, left basal forebrain Ch1–3; RBF1–3, right basal forebrain Ch1–3; BF1–3 Asymmetry, volume difference between the left and right basal forebrain Ch1–3; LBF4, left basal forebrain Ch4; RBF4, right basal forebrain Ch4; BF4 Asymmetry, volume difference between the left and right basal forebrain Ch4.

Table 6. The comparisons of BF subregion volumes in females of three follow–up visits

Characteristics	Baseline (n = 37)	Month–18 (n = 37)	Month–36 (n = 37)	F value	p value	Baseline vs. Month–18	Baseline vs. Month–36	Month–18 vs. Month–36
Age range	61–83	–	–	–	–	–	–	–
MMSE	29.05 ± 0.970	28.84 ± 1.236	28.92 ± 1.090	0.599	0.554	0.904	1.000	1.000
LBF	0.239 ± 0.023	0.238 ± 0.022	0.233 ± 0.024	2.970	0.126	1.000	0.247	0.540
RBF	0.173 ± 0.017	0.172 ± 0.018	0.167 ± 0.016	8.576	*0.009*	1.000	*0.036*	*0.036*
BF Asymmetry	0.322 ± 0.103	0.322 ± 0.091	0.327 ± 0.102	0.118	0.907	1.000	1.000	1.000
LBF1–3	0.097 ± 0.012	0.098 ± 0.010	0.098 ± 0.011	0.098	0.907	1.000	1.000	1.000
RBF1–3	0.063 ± 0.011	0.062 ± 0.010	0.060 ± 0.009	4.751	*0.042*	1.000	0.223	0.247
BF1–3 Asymmetry	0.440 ± 0.191	0.450 ± 0.186	0.479 ± 0.199	2.100	0.203	1.000	0.528	0.950
LBF4	0.142 ± 0.016	0.140 ± 0.017	0.135 ± 0.017	5.948	*0.027*	1.000	*0.036*	0.247
RBF4	0.110 ± 0.011	0.110 ± 0.011	0.107 ± 0.011	2.819	0.126	1.000	0.368	0.540
BF4 Asymmetry	0.247 ± 0.112	0.239 ± 0.090	0.227 ± 0.104	0.504	0.780	1.000	1.000	1.000

Data was presented as mean ± standard deviation. All p values were calculated by using linear mixed effect model. p values corrected by False Discovery Rate (FDR) correction were considered statistically significant and represented as bold and slant bodies.
n, the number of subjects; MMSE, Mini–Mental State Examination; LBF, left basal forebrain; RBF, right basal forebrain; BF Asymmetry, volume difference between the left and right basal forebrain; LBF1–3, left basal forebrain Ch1–3; RBF1–3, right basal forebrain Ch1–3; BF1–3 Asymmetry, volume difference between the left and right basal forebrain Ch1–3; LBF4, left basal forebrain Ch4; RBF4, right basal forebrain Ch4; BF4 Asymmetry, volume difference between the left and right basal forebrain Ch4.

AUTHOR CONTRIBUTIONS

DECLARATION OF INTERESTS

REFERENCES

1. Mesulam, M.M., Mufson, E.J., Wainer, B.H., and Levey, A.I. (1983). Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience 10, 1185–1201. https://doi.org/10.1016/0306-4522(83)90108-2.

2. Wolf, D., Grothe, M., Fischer, F.U., Heinsen, H., Kilimann, I., Teipel, S., and Fellgiebel, A. (2014). Association of basal forebrain volumes and cognition in normal aging. Neuropsychologia 53, 54–63. https://doi.org/10.1016/j.neuropsychologia.2013.11.002.

3. Berger-Sweeney, J. (2003). The cholinergic basal forebrain system during development and its influence on cognitive processes: important questions and potential answers. Neurosci. Biobehav. Rev. 27, 401–411. https://doi.org/10.1016/s0149-7634(03)00070-8.

4. Mufson, E.J., Ginsberg, S.D., Ikonomovic, M.D., and DeKosky, S.T. (2003). Human cholinergic basal forebrain: chemoanatomy and neurologic dysfunction. J. Chem. Neuroanat. 26, 233–242. https://doi.org/10.1016/s0891-0618(03)00068-1.

5. Rasool, C.G., Svendsen, C.N., and Selkoe, D.J. (1986). Neurofibrillary degeneration of cholinergic and noncholinergic neurons of the basal forebrain in Alzheimer's disease. Ann. Neurol. 20, 482–488. https://doi.org/10.1002/ana.410200407.

6. Zaborszky, L., and Duque, A. (2000). Local synaptic connections of basal forebrain neurons. Behav. Brain Res. 115, 143–158. https://doi.org/10.1016/s0166-4328(00)00255-2.

7. Kerbler, G.M., Nedelska, Z., Fripp, J., Laczó, J., Vyhnalek, M., Lisý, J., Hamlin, A.S., Rose, S., Hort, J., and Coulson, E.J. (2015). Basal Forebrain Atrophy Contributes to Allocentric Navigation Impairment in Alzheimer's Disease Patients. Front. Aging Neurosci. 7, 185. https://doi.org/10.3389/fnagi.2015.00185.

8. Chen, X., Farrell, M.E., Rundle, M.M., Chan, M.Y., Moore, W., Wig, G.S., and Park, D.C. (2021). The relationship of functional hippocampal activity, amyloid deposition, and longitudinal memory decline to memory complaints in cognitively healthy older adults. Neurobiol. Aging 105, 318–326. https://doi.org/10.1016/j.neurobiolaging.2021.04.020.

9. Kerbler, G.M., Fripp, J., Rowe, C.C., Villemagne, V.L., Salvado, O., Rose, S., and Coulson, E.J.; Alzheimer's Disease Neuroimaging Initiative (2015). Basal forebrain atrophy correlates with amyloid beta burden in Alzheimer's disease. Neuroimage. Clin. 7, 105–113. https://doi.org/10.1016/j.nicl.2014.11.015.

10. Schmitz, T.W., and Zaborszky, L. (2021). Spatial topography of the basal forebrain cholinergic projections: Organization and vulnerability to degeneration. Handb. Clin. Neurol. 179, 159–173. https://doi.org/10.1016/B978-0-12-819975-6.00008-X.

11. Hall, A.M., Moore, R.Y., Lopez, O.L., Kuller, L., and Becker, J.T. (2008). Basal forebrain atrophy is a presymptomatic marker for Alzheimer's disease. Alzheimers Dement. 4, 271–279. https://doi.org/10.1016/j.jalz.2008.04.005.

12. Teipel, S.J., Flatz, W.H., Heinsen, H., Bokde, A.L.W., Schoenberg, S.O., Stöckel, S., Dietrich, O., Reiser, M.F., Möller, H.J., and Hampel, H. (2005). Measurement of basal forebrain atrophy in Alzheimer's disease using MRI. Brain 128, 2626–2644. https://doi.org/10.1093/brain/awh589.

13. Kilimann, I., Hausner, L., Fellgiebel, A., Filippi, M., Würdemann, T.J., Heinsen, H., and Teipel, S.J. (2017). Parallel Atrophy of Cortex and Basal Forebrain Cholinergic System in Mild Cognitive Impairment. Cereb. Cortex 27, 1841–1848. https://doi.org/10.1093/cercor/bhw019.

14. Grothe, M., Zaborszky, L., Atienza, M., Gil-Neciga, E., Rodriguez-Romero, R., Teipel, S.J., Amunts, K., Suarez-Gonzalez, A., and Cantero, J.L. (2010). Reduction of basal forebrain cholinergic system parallels cognitive impairment in patients at high risk of developing Alzheimer's disease. Cereb. Cortex 20, 1685–1695. https://doi.org/10.1093/cercor/bhp232.

15. Nebel, R.A., Aggarwal, N.T., Barnes, L.L., Gallagher, A., Goldstein, J.M., Kantarci, K., Mallampalli, M.P., Mormino, E.C., Scott, L., Yu, W.H., et al. (2018). Understanding the impact of sex and gender in Alzheimer's disease: A call to action. Alzheimers Dement. 14, 1171–1183. https://doi.org/10.1016/j.jalz.2018.04.008.

16. Laws, K.R., Irvine, K., and Gale, T.M. (2018). Sex differences in Alzheimer's disease. Curr. Opin. Psychiatry 31, 133–139. https://doi.org/10.1097/yco.0000000000000401.

17. Koran, M.E.I., Wagener, M., and Hohman, T.J.; Alzheimer's Neuroimaging Initiative (2017). Sex differences in the association between AD biomarkers and cognitive decline. Brain Imaging Behav. 11, 205–213. https://doi.org/10.1007/s11682-016-9523-8.

18. Sampedro, F., Vilaplana, E., de Leon, M.J., Alcolea, D., Pegueroles, J., Montal, V., Carmona-Iragui, M., Sala, I., Sánchez-Saudinos, M.B., Antón-Aguirre, S., et al. (2015). APOE-by-sex interactions on brain structure and metabolism in healthy elderly controls. Oncotarget 6, 26663–26674. https://doi.org/10.18632/oncotarget.5185.

19. Ardekani, B.A., Convit, A., and Bachman, A.H. (2016). Analysis of the MIRIAD Data Shows Sex Differences in Hippocampal Atrophy Progression. J. Alzheimers Dis. 50, 847–857. https://doi.org/10.3233/JAD-150780.

20. Hua, X., Hibar, D.P., Lee, S., Toga, A.W., Jack, C.R., Jr., Weiner, M.W., and Thompson, P.M.; Alzheimer's Disease Neuroimaging Initiative (2010). Sex and age differences in atrophic rates: an ADNI study with n=1368 MRI scans. Neurobiol. Aging 31, 1463–1480. https://doi.org/10.1016/j.neurobiolaging.2010.04.033.

21. Counts, S.E., Che, S., Ginsberg, S.D., and Mufson, E.J. (2011). Gender differences in neurotrophin and glutamate receptor expression in cholinergic nucleus basalis neurons during the progression of Alzheimer's disease. J. Chem. Neuroanat. 42, 111–117. https://doi.org/10.1016/j.jchemneu.2011.02.004.

22. Gibbs, R.B. (2000). Effects of gonadal hormone replacement on measures of basal forebrain cholinergic function. Neuroscience 101, 931–938. https://doi.org/10.1016/s0306-4522(00)00433-4.

23. Ishunina, T.A., Fisser, B., and Swaab, D.F. (2002). Sex differences in androgen receptor immunoreactivity in basal forebrain nuclei of elderly and Alzheimer patients. Exp. Neurol. 176, 122–132. https://doi.org/10.1006/exnr.2002.7907.

24. Kim, S., Barad, Z., Cheong, R.Y., and Ábrahám, I.M. (2020). Sex differences in rapid nonclassical action of 17beta-oestradiol on intracellular signalling and oestrogen receptor alpha expression in basal forebrain cholinergic neurones in mouse. J. Neuroendocrinol. 32, e12830. https://doi.org/10.1111/jne.12830.

25. Chiesa, P.A., Cavedo, E., Grothe, M.J., Houot, M., Teipel, S.J., Potier, M.C., Habert, M.O., Lista, S., Dubois, B., and Hampel, H.; INSIGHT-preAD Study Group and the Alzheimer Precision Medicine Initiative APMI (2019). Relationship between Basal Forebrain Resting-State Functional Connectivity and

Brain Amyloid-beta Deposition in Cognitively Intact Older Adults with Subjective Memory Complaints. Radiology 290, 167–176. https://doi.org/10.1148/radiol.2018180268.

26. Pombero, A., Bueno, C., Saglietti, L., Rodenas, M., Guimera, J., Bulfone, A., and Martinez, S. (2011). Pallial origin of basal forebrain cholinergic neurons in the nucleus basalis of Meynert and horizontal limb of the diagonal band nucleus. Development 138, 4315–4326. https://doi.org/10.1242/dev.069534.

27. Grothe, M., Heinsen, H., and Teipel, S.J. (2012). Atrophy of the cholinergic Basal forebrain over the adult age range and in early stages of Alzheimer's disease. Biol. Psychiatry 71, 805–813. https://doi.org/10.1016/j.biopsych.2011.06.019.

28. Pini, L., Pievani, M., Bocchetta, M., Altomare, D., Bosco, P., Cavedo, E., Galluzzi, S., Marizzoni, M., and Frisoni, G.B. (2016). Brain atrophy in Alzheimer's Disease and aging. Ageing Res. Rev. 30, 25–48. https://doi.org/10.1016/j.arr.2016.01.002.

29. Brueggen, K., Dyrba, M., Barkhof, F., Hausner, L., Filippi, M., Nestor, P.J., Hauenstein, K., Klöppel, S., Grothe, M.J., Kasper, E., and Teipel, S.J. (2015). Basal Forebrain and Hippocampus as Predictors of Conversion to Alzheimer's Disease in Patients with Mild Cognitive Impairment - A Multicenter DTI and Volumetry Study. J. Alzheimers Dis. 48, 197–204. https://doi.org/10.3233/JAD-150063.

30. Kilimann, I., Grothe, M., Heinsen, H., Alho, E.J.L., Grinberg, L., Amaro, E., Jr., Dos Santos, G.A.B., da Silva, R.E., Mitchell, A.J., Frisoni, G.B., et al. (2014). Subregional basal forebrain atrophy in Alzheimer's disease: a multicenter study. J. Alzheimers Dis. 40, 687–700. https://doi.org/10.3233/JAD-132345.

31. Herdick, M., Dyrba, M., Fritz, H.C.J., Altenstein, S., Ballarini, T., Brosseron, F., Buerger, K., Can Cetindag, A., Dechent, P., Dobisch, L., et al. (2020). Multimodal MRI analysis of basal forebrain structure and function across the Alzheimer's disease spectrum. Neuroimage. Clin. 28, 102495. https://doi.org/10.1016/j.nicl.2020.102495.

32. Hohmann, C.F., and Berger-Sweeney, J. (1998). Sexually dimorphic responses to neonatal basal forebrain lesions in mice: II. Cortical morphology. J. Neurobiol. 37, 595–606.

33. Gibbs, R.B. (1998). Impairment of Basal Forebrain Cholinergic Neurons Associated with Aging and Long-Term Loss of Ovarian Function. Exp. Neurol. 151, 289–302. https://doi.org/10.1006/exnr.1998.6789.

34. Cantero, J.L., Zaborszky, L., and Atienza, M. (2017). Volume Loss of the Nucleus Basalis of Meynert is Associated with Atrophy of Innervated Regions in Mild Cognitive Impairment. Cereb. Cortex 27, 3881–3889. https://doi.org/10.1093/cercor/bhw195.

35. Duboc, V., Dufourcq, P., Blader, P., and Roussigné, M. (2015). Asymmetry of the Brain: Development and Implications. Annu. Rev. Genet. 49, 647–672. https://doi.org/10.1146/annurev-genet-112414-055322.

36. Kong, X.Z., Postema, M.C., Guadalupe, T., de Kovel, C., Boedhoe, P.S.W., Hoogman, M., Mathias, S.R., van Rooij, D., Schijven, D., Glahn, D.C., et al. (2022). Mapping brain asymmetry in health and disease through the ENIGMA consortium. Hum. Brain Mapp. 43,

167–181. https://doi.org/10.1002/hbm.25033.

37. Crow, T.J., Chance, S.A., Priddle, T.H., Radua, J., and James, A.C. (2013). Laterality interacts with sex across the schizophrenia/bipolarity continuum: an interpretation of meta-analyses of structural MRI. Psychiatry Res. 210, 1232–1244. https://doi.org/10.1016/j.psychres.2013.07.043.

38. Bruder, G.E., Stewart, J.W., Hellerstein, D., Alvarenga, J.E., Alschuler, D., and McGrath, P.J. (2012). Abnormal functional brain asymmetry in depression: evidence of biologic commonality between major depression and dysthymia. Psychiatry Res. 196, 250–254. https://doi.org/10.1016/j.psychres.2011.11.019.

39. Ishikawa, W., Sato, M., Fukuda, Y., Matsumoto, T., Takemura, N., and Sakatani, K. (2014). Correlation between asymmetry of spontaneous oscillation of hemodynamic changes in the prefrontal cortex and anxiety levels: a near-infrared spectroscopy study. J. Biomed. Opt. 19, 027005. https://doi.org/10.1117/1.JBO.19.2.027005.

40. Zach, P., Vales, K., Stuchlik, A., Cermakova, P., Mrzilkova, J., Koutela, A., and Kutova, M. (2016). Effect of stress on structural brain asymmetry. Neuro Endocrinol Lett. 37, 253–264.

41. Wang, C., Wei, Y., Li, J., Li, X., Liu, Y., Hu, Q., and Wang, Y.; Alzheimer's Disease Neuroimaging Initiative (2022). Asymmetry-enhanced attention network for Alzheimer's diagnosis with structural Magnetic Resonance Imaging. Comput. Biol. Med. 151, 106282. https://doi.org/10.1016/j.compbiomed.2022.106282.

42. Derflinger, S., Sorg, C., Gaser, C., Myers, N., Arsic, M., Kurz, A., Zimmer, C., Wohlschläger, A., and Mühlau, M. (2011). Grey-matter atrophy in Alzheimer's disease is asymmetric but not lateralized. J. Alzheimers Dis. 25, 347–357. https://doi.org/10.3233/JAD-2011-110041.

43. Toga, A.W., and Thompson, P.M. (2003). Mapping brain asymmetry. Nat. Rev. Neurosci. 4, 37–48. https://doi.org/10.1038/nrn1009.

44. Nazlee, N., Waiter, G.D., and Sandu, A.L. (2022). Age-associated sex and asymmetry differentiation in hemispheric and lobar cortical ribbon complexity across adulthood: A UK Biobank imaging study. Hum. Brain Mapp. 44, 49–65. https://doi.org/10.1002/hbm.26076.

45. Liang, X., Zhao, C., Jin, X., Jiang, Y., Yang, L., Chen, Y., and Gong, G. (2021). Sex-related human brain asymmetry in hemispheric functional gradients. Neuroimage 229, 117761. https://doi.org/10.1016/j.neuroimage.2021.117761.

46. Amunts, V.V. (2006). Structural asymmetry of the basal nucleus of Meynert in men and women. Zh Nevrol Psikhiatr 106, 50–54.

47. Kőszegi, Z., and Abrahám, I.M. (2012). Effect of ageing on post-lesion oestradiol treatment on mouse cholinergic neurones in vivo. J. Neuroendocrinol. 24, 1243–1248. https://doi.org/10.1111/j.1365-2826.2012.02331.x.

48. Chaves-Coira, I., Garcia-Magro, N., Zegarra-Valdivia, J., Torres-Aleman, I., and Nunez, A. (2023). Cognitive Deficits in Aging Related to Changes in Basal Forebrain Neuronal Activity. Cells 12, 1477. https://doi.org/10.3390/cells12111477.

49. Gogniat, M.A., Robinson, T.L., Mewborn, C.M., Jean, K.R., and Miller, L.S. (2018). Body

mass index and its relation to neuropsychological functioning and brain volume in healthy older adults. Behav. Brain Res. 348, 235–240. https://doi.org/10.1016/j.bbr.2018.04.029.

50. Ngwa, J.S., Fungwe, T.V., Ntekim, O., Allard, J.S., Johnson, S.M., Castor, C., Graham, L., Nadarajah, S., Gillum, R.F., and Obisesan, T.O.; Alzheimer's Disease Neuroimaging Initiative (2018). Associations of Pulse and Blood Pressure with Hippocampal Volume by APOE and Cognitive Phenotype: The Alzheimer's Disease Neuroimaging Initiative (ADNI). Dement. Geriatr. Cogn. Disord 45, 66–78. https://doi.org/10.1159/000486955.

51. Pedrini, S., Doecke, J.D., Hone, E., Wang, P., Thota, R., Bush, A.I., Rowe, C.C., Dore, V., Villemagne, V.L., Ames, D., et al. (2022). Plasma high-density lipoprotein cargo is altered in Alzheimer's disease and is associated with regional brain volume. J. Neurochem. 163, 53–67. https://doi.org/10.1111/jnc.15681.

52. Downer, B., Jiang, Y., Zanjani, F., and Fardo, D. (2015). Effects of alcohol consumption on cognition and regional brain volumes among older adults. Am. J. Alzheimers Dis. Other Demen. 30, 364–374. https://doi.org/10.1177/1533317514549411.

53. Ellis, K.A., Bush, A.I., Darby, D., De Fazio, D., Foster, J., Hudson, P., Lautenschlager, N.T., Lenzo, N., Martins, R.N., Maruff, P., et al. (2009). The Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging: methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer's disease. Int. Psychogeriatr. 21, 672–687. https://doi.org/10.1017/s1041610209009405.

54. Petersen, R.C., Smith, G.E., Waring, S.C., Ivnik, R.J., Tangalos, E.G., and Kokmen, E. (1999). Mild cognitive impairment: clinical characterization and outcome. Arch. Neurol. 56, 303–308. https://doi.org/10.1001/archneur.56.3.303.

55. Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L.O., Nordberg, A., Bäckman, L., Albert, M., Almkvist, O., et al. (2004). Mild cognitive impairment—beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J. Intern. Med. 256, 240–246. https://doi.org/10.1111/j.1365-2796.2004.01380.x.

56. McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E.M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34, 939–944. https://doi.org/10.1212/wnl.34.7.939.

57. Zaborszky, L., Hoemke, L., Mohlberg, H., Schleicher, A., Amunts, K., and Zilles, K. (2008). Stereotaxic probabilistic maps of the magnocellular cell groups in human basal forebrain. Neuroimage 42, 1127–1141. https://doi.org/10.1016/j.neuroimage.2008.05.055.

58. Eickhoff, S.B., Stephan, K.E., Mohlberg, H., Grefkes, C., Fink, G.R., Amunts, K., and Zilles, K. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. Neuroimage 25, 1325–1335. https://doi.org/10.1016/j.neuroimage.2004.12.034.

59. Manjón, J.V., and Coupé, P. (2016). volBrain: An Online MRI Brain Volumetry System. Front. Neuroinform. *10*, 30. https://doi.org/10.3389/fninf.2016.00030.

60. Fowler, C., Rainey-Smith, S.R., Bird, S., Bomke, J., Bourgeat, P., Brown, B.M., Burnham, S.C., Bush, A.I., Chadunow, C., Collins, S., et al. (2021). Fifteen Years of the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study: Progress and Observations from 2,359 Older Adults Spanning the Spectrum from Cognitive Normality to Alzheimer's Disease. J. Alzheimers Dis. Rep. *5*, 443–468. https://doi.org/10.3233/ADR-210005.

61. Yu, M., Linn, K.A., Cook, P.A., Phillips, M.L., McInnis, M., Fava, M., Trivedi, M.H., Weissman, M.M., Shinohara, R.T., and Sheline, Y.I. (2018). Statistical harmonization corrects site effects in functional connectivity measurements from multi-site fMRI data. Hum. Brain Mapp. *39*, 4213–4227. https://doi.org/10.1002/hbm.24241.

62. Albaugh, M.D., Ducharme, S., Karama, S., Watts, R., Lewis, J.D., Orr, C., Nguyen, T.-V., McKinstry, R.C., Botteron, K.N., Evans, A.C., et al. (2017). Anxious/depressed symptoms are related to microstructural maturation of white matter in typically developing youths. Dev. Psychopathol. *29*, 751–758. https://doi.org/10.1017/s0954579416000444.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
The Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Aging	Kathryn A Ellis et al.; Int Psychogeriatr. 2009 Aug; 21(4):672-87. https://doi.org/10.1017/S1041610209009405.	https://aibl.org.au/
Software and algorithms		
Computational Anatomy Toolbox (CAT 12)	NeuroImaging Tools & Resources Collaboratory	https://neuro-jena.github.io/cat/
Statistical Parametric Mapping (SPM 12)	https://github.com/spm/	https://www.fil.ion.ucl.ac.uk/spm/
Statistical Product and Service Solutions (SPSS 25.0)	The IBM SPSS software platform	https://www.ibm.com/spss

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Guanghui Yu (ghyu@sdfmu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Aging dataset is available at: https://aibl.org.au/.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The Australian Imaging, Biomarkers and Lifestyle (AIBL) Flagship Study of Aging (https://aibl.org.au/) is a prospective longitudinal study that conducts follow–up assessments at 18–months intervals.[53] This study focuses on early detection and lifestyle interventions, encompassing patients with AD and MCI as well as HCs. The participants were long-term residents of Melbourne and Perth, Australia, with the majority being of Caucasian, but the AIBL study does not collect information regarding race or ethnicity. Exclusion criteria for the present study included participants with schizophrenia, bipolar disorder, Parkinson's disease, symptomatic stroke, dementia other than AD, a Geriatric Depression Score (GDS) of 5 or higher, psychiatric illnesses, cancer, uncontrolled diabetes, a history of head injury with over 1 h of post-traumatic amnesia, a history of alcoholism, and contraindications for MRI scanning.

In the cross–sectional study, 221 participants (AD = 71, MCI = 73, HCs = 77) were recruited from AIBL. The MCI group was defined by the International Working Group on Mild Cognitive Impairment criteria,[54,55] and AD patients were defined by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria.[56] The longitudinal study involved 77 cognitively normal older adults (40 males and 37 females) from AIBL who were aged between 61 and 86 years at baseline. All participants had the same number of follow–up visits, the baseline, month–18, and month–36, and they all successfully completed the assessments and remained cognitively normal at each visit. It's worth noting that the subjects in the longitudinal study were identical to the HCs in the cross-sectional study.

All of the subjects signed consent forms to undergo MRI and neuropsychological assessment for clinical investigation and research. This study was approved by the institutional ethics committees of Austin Health, St Vincent's Health, Hollywood Private Hospital, and Edith Cowan University.

METHOD DETAILS

MRI data acquisition method details

T$_1$–weighted images were acquired on Siemens 3.0 T MR scanner (Trio/Skyra/Verio). All of the participants received an MRI scan using a three–dimensional magnetization–prepared rapid acquisition gradient–echo (3D–MPRAGE) sequence. The parameters were as follows: repetition

time (TR) = 2300 ms; echo time (TE) = 2.98 ms; inversion time (TI) = 900 ms; flip angle = 9°; field of view (FOV) = 240 mm × 256 mm; slice thickness = 1.2 mm; axial slices = 160; voxel size = 1 mm × 1 mm × 1.2 mm.

Image processing and volume estimation of BF

Structural MRI images were preprocessed using an automated program within the Computational Anatomy Toolbox (CAT12, https://neuro-jena.github.io/cat/) in Statistical Parametric Mapping (SPM12, https://www.fil.ion.ucl.ac.uk/spm/). All of the individual data were processed by: 1) skull–stripping of the brain; 2) a spatial adaptive nonlocal means denoising filter; 3) bias correction; 4) affine registration; and 5) alignment of T_1–weighted images with the anterior and posterior commissure line on the sagittal plane, followed by segmentation into GM, white matter (WM), and CSF. The volume of BF cholinergic nuclei, including Ch1–Ch3 and Ch4, was further calculated using cytoarchitecture probabilistic maps of compartments of the BF magnocellular system[57] in the Anatomy toolbox in SPM.[58] In addition, the total intracranial volume (TIV) was calculated for statistical analysis.

QUANTIFICATION AND STATISTICAL ANALYSIS

The volumes of the left basal forebrain (LBF), right basal forebrain (RBF), LBF Ch1–Ch3 (LBF1–3), RBF Ch1–Ch3 (RBF1–3), LBF Ch4 (LBF4), and RBF Ch4 (RBF4) were obtained in the previous step. The asymmetry indices of BF, BF1–3, and BF4 were also calculated as the difference between the right and left volumes divided by their mean (in percent).[59] Statistical analyses were performed with SPSS (version 25.0, Armonk, NY, USA). Data were expressed as mean ± standard deviation. Taking into account that MRI scans were acquired at three different scanning centers, with two in Melbourne utilizing Siemens 3T Trio and Siemens 3T Skyra scanners, and one in Perth using Siemens 3T Verio scanner,[60] we further employed an empirical Bayes approach known as ComBat to remove scanner effects.[61]

Cross–sectional study

The Pearson Chi–square test was employed to compare the categorical variable (sex). One–way analysis of variance (ANOVA) was utilized to analyze the differences in continuous variables such as age and Mini–Mental State Examination (MMSE) scores. The significance level was set at $p < 0.05$. When comparing the volumes of BF subregion, we first normalized the effect of TIV by dividing the volume of each brain region by its corresponding TIV. Subsequently, one–way analysis of covariance (ANCOVA) was applied to compare the differences among the three groups, with age as covariates. Post hoc analysis was conducted using a two–sample t test. In addition, we assessed the interaction effect between group and sex. The p values were considered to be significant after the Benjamini and Hochberg (False Discovery Rate, FDR) correction for the multiple comparison correction. The significance level was set at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p \leq 0.001$).

Longitudinal study

Given the potential correlations and interdependencies between brain regions volumes at each follow–up, longitudinal data were investigated using Linear mixed-effects models, which can effectively analyze unbalanced longitudinal data and maximize statistical power.[62] The volume of BF subregion was considered the response variable, subject ID was included as a random effect, and both sex and follow–up time were entered as fixed effects. Additionally, we evaluated the interaction effect between time and sex. We conducted pairwise comparisons to assess the differences between two follow–up visits. The p values were considered to be significant after FDR correction for multiple comparisons, and the significance level was set at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p \leq 0.001$).