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Atrophy of hippocampal subfield CA2/3 in healthy elderly men is related to educational attainment

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ABSTRACT

A higher education level is a protective factor against cognitive decline in elders; however, the underlying neural mechanisms remain unclear. Modulated by both aging and education, the hippocampus is a starting point for understanding the long-lasting effect of education on the aging of human brain. Because the hippocampus possesses functionally heterogeneous subfields and exhibits sex differences, we examined hippocampal subfields in men and women separately. We performed both cross-sectional (n = 143) and longitudinal (n = 51) analyses on healthy participants aged 65—75 years, who underwent structural magnetic resonance imaging. Volumes of the hippocampi and their subfields were estimated by automated segmentation. We found significantly positive correlations between educational attainment and the volume of hippocampal CA2/3 in men but not in women. The longitudinal analysis focusing on this region validated the above results by showing that a higher education level attenuated the progression of atrophy during a 15-month follow-up period in the CA2/3 region in men. These findings suggest that, in men, education plays a role in the aging of specific hippocampal subfields.

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1. Introduction

Education is an important moderator of cognitive decline and dementia. Several prior studies have related a higher education level to a lower risk of cognitive decline and clinical dementia (Lee et al., 2003; Mortimer et al., 2003; Qiu et al., 2001), with the risk of dementia being reduced by 7% for each additional year of education (Xu et al., 2016). This suggests that a higher education level may improve late-life quality by preventing cognitive decline and dementia, although the potential mechanisms remain unclear (Bento-Torres et al., 2017; Le Carret et al., 2003; Reuser et al., 2011; Shpanskaya et al., 2014).

The hippocampus plays key roles in learning, memory, and spatial navigation (Astur et al., 2002; Han et al., 2016), and the changes that occur in this region during aging are closely related to the preservation of cognitive functions (Elcombe et al., 2015; Ezzati et al., 2016; Jiang et al., 2014; Wei et al., 2018). Some studies have demonstrated that education exerts protective effects on the aging process of the hippocampus (Noble et al., 2012; Piras et al., 2011; Valenzuela et al., 2008). In a sample of 150 healthy participants (aged 18—65 years), a significantly negative correlation was observed between the participants' education level and the white matter mean diffusivity (MD) in the bilateral hippocampus, implying that a lower MD in the hippocampus may compensate for cognitive decline (Piras et al., 2011). In adulthood, the decrease in hippocampal volume is less pronounced in highly educated individuals than it is in less educated individuals, suggesting that the age-related decline of hippocampal volume may be buffered by educational attainment (Noble et al., 2012). Another study showed that higher levels of education are associated with reduced rates of hippocampal atrophy under normal aging conditions (Valenzuela et al., 2008). On the contrary, some studies have failed to reveal any effects of education on hippocampal volume (Liu et al., 2012; Shpanskaya et al., 2014). Thus, more research is needed to elucidate the relationship between education and age-related changes in hippocampal volume.

Two characteristics of the hippocampus particularly signal the need for more thorough investigations with regard to the effects of education on the normal aging processes of the hippocampus. First, the hippocampus consists of various subfields that are associated with different cognitive functions (Beyer et al., 2013; Ezzati et al., 2014; Yassa et al., 2010). Individuals with amnestic mild cognitive impairment have smaller CA1 and dentate gyrus (DG)/CA3 regions (Yassa et al., 2010); furthermore, delayed free recall scores in patients with Parkinson's disease are positively correlated with the hippocampal atrophy of the CA1, CA3, and subiculum areas (Beyer et al., 2013). Moreover, hippocampal subfield volumes have been linked to cognitive function in healthy older adults (Shing et al., 2011; Zammit et al., 2017). For instance, Shing et al. (2011) revealed that larger CA3—4 and DG volumes are associated with better associative recognition memory. In another study, Zammit et al. (2017) showed that the volumes of the CA3 and subiculum regions are associated with visual and verbal episodic memory. However, the effects of education on the hippocampal subfield regions are still unclear.

Second, numerous studies have identified sex differences in the volume and volume changes of the hippocampal regions (Crivello et al., 2014; Krogsrud et al., 2014). For instance, Krogsrud et al. (2014) reported that many subfields show smaller volumes in women than in men aged 4—22 years, implying that sex may influence the developmental trajectories of the hippocampus. In a longitudinal community cohort study, women showed marginally significantly higher rates of hippocampal atrophy than did men (Crivello et al., 2014). Moreover, previous studies reported that older women show higher incidence rates of dementia or Alzheimer's disease (AD) than older men (Gao et al., 1998; Neu et al., 2017; Oveisgharan et al., 2018).

Considering the above characteristics, the effects of education on hippocampal aging should be examined further. Therefore, the aim of the present study was to determine whether education level selectively modulates the age-related volumetric changes in the hippocampal subfields and whether there are sex differences in the effects of education. These questions were examined with both cross-sectional (n = 143) and longitudinal (n = 51) analyses utilizing brain magnetic resonance imaging (MRI) scans of the participants aged 65—75 years.

2. Material and methods

2.1. Participants

This study was approved by the Human Research Ethics Committees of Shanghai Mental Health Center (Approval number: 2013-40) and Tongji Hospital in Shanghai (Approval number: LL(H)-09-04), China. Written informed consent was obtained from the participants after the study procedures had been explained to them.

Participants were community-dwelling older adults who were living in the neighborhoods located in the Jingan and Putuo Districts of Shanghai. They were recruited by local neighborhood committees using posters and radio advertisements. All the participants were admitted to the study after a personal interview based on the following inclusion criteria: aged 65—75 years; >1 year of formal education; independently living in the community; no disabilities; no difficulties in hearing, vision, or communication; no severe physical diseases or psychotic disorders; and a score on the Chinese version of the Mini-Mental State Examination (CMMSE) ≥19 for elementary education and ≥24 for middle school education and above; the normal cutoff point of the MMSE score is lower because of the lower educational level in China (Li et al., 2006). Exclusion criteria were as follows: obvious cognitive decline; a diagnosis of Alzheimer's disease; major neurological and/or psychiatric disorders such as brain cancer, cerebral infarction, cerebral hemorrhage, malnutrition, major depressive disorder, and schizophrenia.

Flow charts of the selection and inclusion processes for participants during the entire study are illustrated in Fig. 1. Among the 539 eligible individuals who were contacted for participation from November 2013 to September 2014, 175 were included in the MRI subsample. Among them, 151 individuals subsequently completed the MRI scans, and 143 participants were finally included in the cross-sectional analysis. Eight participants were excluded from the statistical analyses because of excessive head movement (n = 3) or
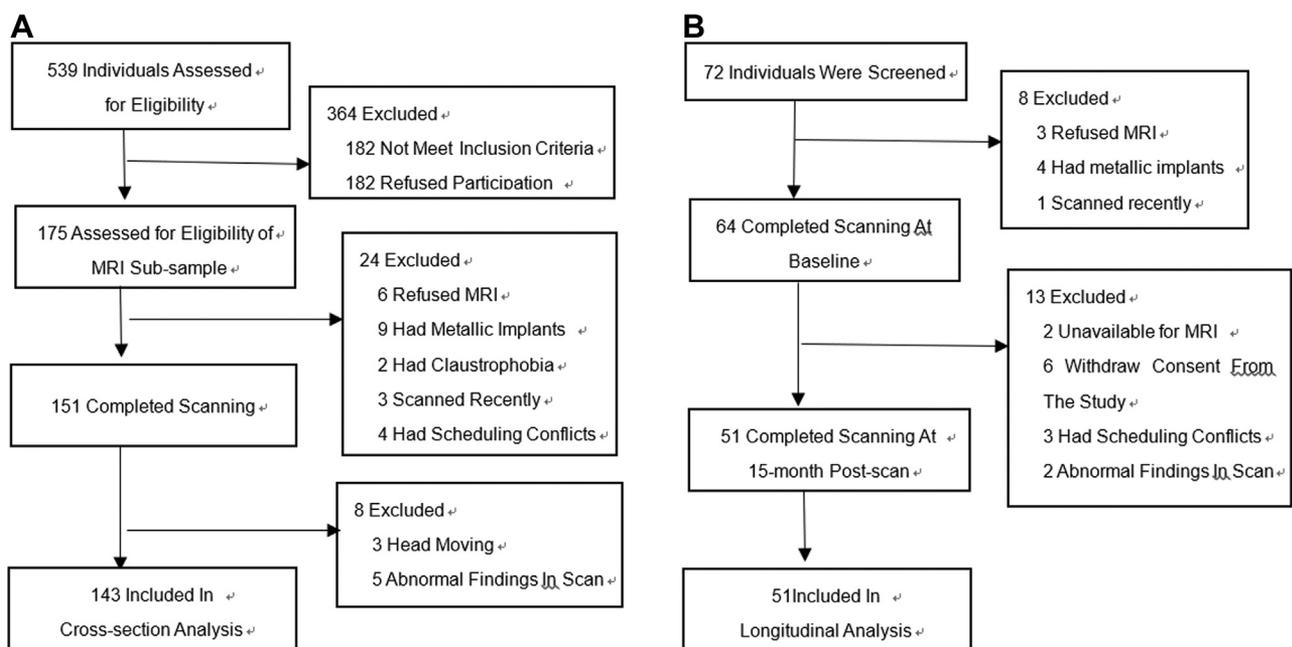

Fig. 1. Flow charts illustrating the process of study participant inclusion. (A) Flow chart for the cross-sectional sample. (B) Flow chart for the longitudinal sample.

abnormal MRI findings (n = 5) (Fig. 1A). With regard to the longitudinal analysis, 64 participants underwent baseline MRI examinations, whereas 51 participants underwent the follow-up MRI examination after 15 months. A total of 13 participants withdrew from the follow-up because of unavailability (n = 2), withdrawn consent (n = 6), scheduling conflicts (n = 3), or abnormal MRI findings (n = 2) (Fig. 1B).

2.2. Cognitive measurement

The Repeatable Battery for the Assessment of Neuropsychological Status ([RBANS], Form A) (Randolph et al., 1998), which has been shown to have good reliability and validity in a sample of Chinese community-living older people, was used to evaluate the specific domains of cognitive function in the participants (Cheng et al., 2011). It consists of 12 subtests, which produce 5 age-adjusted index scores for the following cognitive abilities: immediate memory, visuospatial/constructional, language, attention, and delayed memory, in addition to a total cognitive functional score. Trained research assistants conducted all cognitive assessments according to the protocol detailed in the manual.

2.3. MRI acquisition

We performed MRI on 2 Siemens 3.0-Tesla scanners (Siemens Medical, Erlangen, Germany), including a Siemens Magnetom Verio (scanner 1) and Siemens Tim Trio (scanner 2) scanner. Images were obtained using a standard 12-channel head coil. Foam padding was used to minimize head motion for each participant. All participants underwent the same structural scanning protocol, which included high-resolution T1-weighted three-dimensional magnetization prepared rapid-acquisition gradient echo imaging in the sagittal plane (repetition time = 1900 ms, echo time = 3.43 ms, flip angle = 9°, matrix size = 256 × 256, field of view = 240 × 240 mm^2, slice thickness = 1 mm, voxel size = 0.9 × 0.9 × 1.0 mm^3, and 160 slices; each scan was 5 minutes in duration). All 143 scans used in the cross-sectional analysis were acquired on scanner 1. Among the 51 participants in the longitudinal study, 37 and 14 were scanned on scanners 1 and 2, respectively. The interval between the baseline and follow-up scans was 15 months. Images were reconstructed and visually checked for major artifacts (e.g., motion, ringing, wrap around, and neurological abnormalities) before further processing.

2.4. Image preprocessing

Structural MR images were processed using the FreeSurfer 6.0 image analysis suit (http://surfer.nmr.mgh.harvard.edu/). The entire hippocampal formation was segmented using FreeSurfer's standard segmentation procedure (Fischl et al., 2002). Briefly, the processing stream includes motion correction and averaging of the 2 T1-weighted volumes, removal of nonbrain tissue using a hybrid watershed/surface deformation procedure (Segonne et al., 2004), automated transformation to the Talairach reference space, and segmentation of the subcortical white matter and deep gray matter volumetric structures using a probabilistic brain atlas (Fischl et al., 2002). All longitudinal images were processed using FreeSurfer's longitudinal pipeline. The procedures for longitudinal processing have been described previously (Jiang et al., 2016). Further, automated segmentation of the hippocampal subfields was implemented in FreeSurfer 6.0, which was developed for more-precise hippocampal subfield segmentation (Wisse et al., 2014). The new version of FreeSurfer (version 6.0) used a hippocampal subfield atlas that was built from a combination of 15 ultra-high-resolution, ex vivo, 0.13-mm isotropic resolution MR images and an independent data set of 39 in vivo, 1-mm isotropic resolution MR images (Iglesias et al., 2015). The statistical atlas constructed from the ultra-high-resolution ex vivo MR images was used to estimate the volume of the hippocampal subfields. The following 13 subfield volumes were calculated for each side of the hippocampus: CA1, CA2/3, CA4, granule cell layer of the dentate gyrus, fimbria, subiculum, presubiculum, parasubiculum, molecular layer, hippocampus-amygdala transition area (HATA), hippocampal tail, hippocampal fissure, and whole hippocampus. Fig. 2 shows the hippocampal subfield segmentation results in one of the participants. The segmentation results were visually inspected for errors in all data sets, and no manual edits were performed.

To account for differences in head size, we used the estimated total intracranial volume (eTIV) to adjust the subfield volumes in the subsequent statistical analysis. Raw volumes were corrected for the eTIV via the following formula: $Volume_{adji} = (Volume_{rawi} \times$
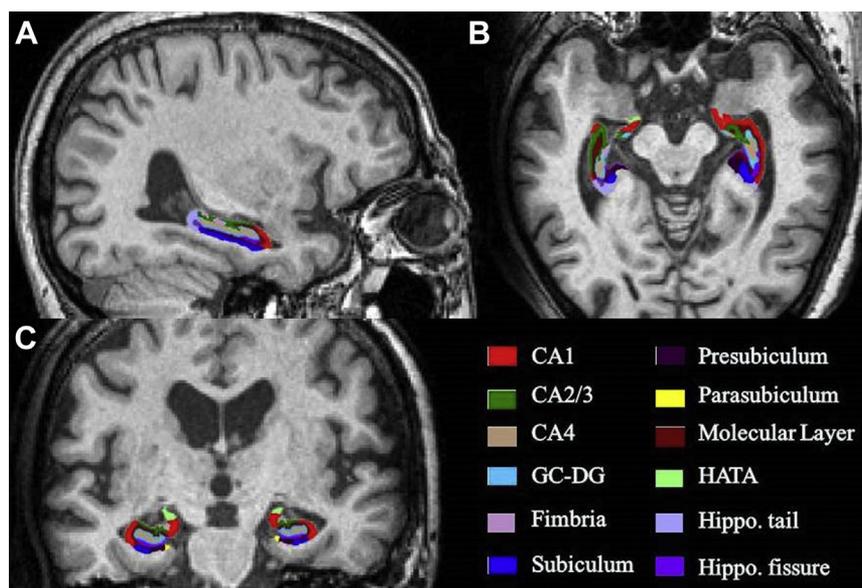

Fig. 2. Automated segmentation of the hippocampal subfields. (A) Sagittal, (B) axial, and (C) coronal magnetic resonance images of the hippocampal subfields from one participant. Abbreviations: CA, cornu ammonis; GC-DG, granule cell layer of the dentate gyrus; HATA, hippocampus-amygdala transition area; Hippo, Hippocampal.

Mean eTIV)/eTIV$_i$, where Volume $_{adji}$ is the adjusted regional volume, Volume $_{rawi}$ is the raw volume for each participant, and Mean eTIV is the sample mean of the eTIV (La Joie et al., 2010). In addition, in the longitudinal analysis, the percentage changes in cognitive measures and hippocampal subfield volumes between the 2 time points for each participant were calculated using the following formula: % change = ([variable $_{baseline}$—variable $_{follow-up}$]/variable $_{baseline}$) × 100.

2.5. Statistical analyses

All statistical analyses were performed using SPSS, version 20 (IBM Corporation, Somer, NY, USA). The statistical significance threshold was set at $p < 0.05$. Mann-Whitney tests were applied to compare demographic variables such as age, education, and CMMSE score between women and men. As there were no significant interactions between education and hemisphere for any of the hippocampal subfield volumes (see Supplementary Tables S1 and S2 for details), the average of the left and right subfield volumes was used for all further statistical analyses. For the cross-sectional analysis, comparisons of eTIV, cognitive performance, and the adjusted volume of the hippocampal subfields between men and women were performed using the general linear model, adjusted for age, education, and CMMSE score. Linear regression models were used to estimate the association between the adjusted volume of the hippocampal subfields and the education level after adjustment for age, sex, and CMMSE score. Moreover, to evaluate whether the association between the adjusted volumes of the hippocampal subfields and the education level was driven by sex differences, we stratified the entire sample by sex and repeated all the previously mentioned models separately for each sex. For the longitudinal analysis, the general linear model was used to evaluate differences in the percentage change of the cognitive measures and hippocampal subfield volumes between the sexes and scanners (scanner 1 and scanner 2). Subsequently, we adopted linear regression models to investigate the associations between the education level and the percentage change in hippocampal subfield volumes, adjusted for age, sex, CMMSE score, and scanner.

3. Results

3.1. Characteristics of study participants and associations between cognitive performance and hippocampal subfield volumes in the cross-sectional sample

Among the 143 individuals included in the cross-sectional analysis, 85 were women and 58 were men, with a mean (standard deviation) age of 68.72 (2.93) years and a mean formal education duration of 12.52 (2.88) years. The mean CMMSE score was 28.09 (1.64). No significant differences were identified between women and men in terms of age, education, and CMMSE score. The RBANS total score and 3 index scores were significantly higher in women than in men, whereas the volume of eTIV was significantly smaller in women than in men (Table 1).

The statistical results of volumetric differences between the men and women are presented in Fig. 3. In the hippocampal subfield analysis, the volumes of CA1 (F = 6.002, p = 0.016), GC-DG (F = 6.518, p = 0.012), fimbria (F = 9.503, p = 0.002), subiculum (F = 6.221, p = 0.014), presubiculum (F = 4.520, p = 0.035), molecular layer (F = 7.645, p = 0.006), HATA (F = 5.964, p = 0.016), and whole hippocampus (F = 6.096, p = 0.015) were significantly smaller in men than in women. Only the CA1, fimbria, subiculum, molecular layer, HATA, and whole hippocampus were significantly smaller in men after false discovery rate correction. However, no significant volume differences were identified between men and women in the CA2/3, CA4, parasubiculum, hippocampal tail, and hippocampal fissure regions.

Partial correlation analysis was used to explore the relationships of RBANS domain index scores with the hippocampal subfield volumes and eTIV in the entire sample, women, and men. After controlling for age, sex, and education, the delayed memory index score was positively associated with the volumes of the CA4 (r = 0.171, p = 0.043) and molecular layer (r = 0.168, p = 0.047) regions in the entire sample, at a significant level. However, there were no associations between RBANS domain index scores and hippocampal subfield volumes in the women and men. In addition, no significant relationships between RBANS domain index scores and eTIV were observed in the entire sample, women, and men.

3.2. Associations between education and hippocampal subfield volumes

As shown in Table 2, after adjustment for age, sex, and CMMSE score in the regression models, education was marginally associated with the adjusted volume of the CA2/3 (Beta = 0.129, p = 0.130) subfield in the entire sample at a significant level.

Considering the heterogeneous sex differences described in the present study and the previous studies that examined the relationship between sex differences in the hippocampal structures and the incidence of AD (Murphy et al., 1996; Neu et al., 2017), we further stratified the analysis by sex. We conducted multiple linear regression analyses with the adjusted volume of the hippocampal subfields as the dependent variable and education, age, and CMMSE score as the independent variables (Table 2). In men, significant positive

Table 1
Demographic and cognitive functional characteristics of the participants included in the cross-sectional analysis

Characteristics	Entire sample (n = 143)	Women (n = 85)	Men (n = 58)	F/Z	p
Age (y)	68.72 ± 2.93	68.40 ± 2.81	69.18 ± 3.05	−1.499	0.134
Education (y)	12.52 ± 2.88	12.39 ± 2.77	12.72 ± 3.04	−0.686	0.493
CMMSE (range 0—30)	28.09 ± 1.64	28.28 ± 1.43	27.81 ± 1.88	−1.242	0.214
RBANS total score	93.96 ± 11.50	95.40 ± 11.19	91.84 ± 11.73	4.297	0.040*
Immediate memory	83.52 ± 13.54	85.65 ± 12.08	80.41 ± 15.01	7.505	0.007*
Visuospatial/Constructional	102.10 ± 16.29	101.01 ± 16.89	103.71 ± 15.38	1.477	0.226
Language	97.53 ± 8.18	98.07 ± 9.39	96.74 ± 5.99	0.726	0.396
Attention	100.80 ± 13.05	102.60 ± 12.28	98.16 ± 13.78	4.565	0.034*
Delayed memory	95.02 ± 13.81	97.15 ± 12.69	91.90 ± 14.87	5.177	0.024*
eTIV (cm^3)	1437.79 ± 182.87	1338.06 ± 134.94	1583.95 ± 141.08	100.636	0.000*

Key: CMMSE, Chinese version of the Mini-Mental State Examination; eTIV, estimated total intracranial volume; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.
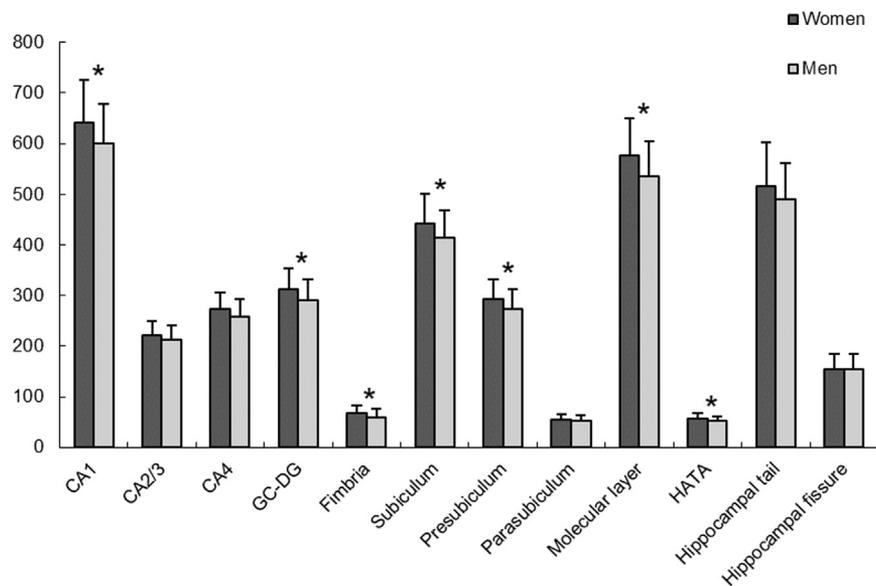Data are presented as mean ± standard deviation.
* $p < 0.05$.

Fig. 3. Comparisons of the adjusted volumes (mm³) of the hippocampal subfields between women and men from the cross-sectional sample. Bar graphs present the distributions of the mean hippocampal subfield volumes among participants. Error bars represent the standard deviation. *p < 0.05. Abbreviations: CA, cornu ammonis; GC-DG, granule cell layer of the dentate gyrus; HATA, hippocampus-amygdala transition area.

correlation between education and hippocampal CA2/3 subfield volume was observed (Beta = 0.263, p = 0.048, uncorrected for multiple comparisons). In contrast, no significant correlations between education and hippocampal subfield volumes were noted in women (p > 0.5). Besides, there was no significant association between education and eTIV in the entire sample (Beta = 0.002, p = 0.976), women (Beta = −0.095, p = 0.424), and men (Beta = 0.105, p = 0.442).

3.3. Characteristics of study participants and associations between cognitive functions and hippocampal subfield volume changes in the longitudinal sample

To validate the findings of the cross-sectional analyses, a total of 51 healthy participants were included in the longitudinal analysis. The participants had a mean (standard deviation) age of 68.94 (3.11)

years, a mean CMMSE score of 28.20 (1.73), and a mean of 11.73 (3.03) years of education. The sample contained 27 women and 24 men. No differences in age, education, and CMMSE score were found between the women and men. Supplementary Table S3 shows the percentage changes in the cognitive functions and hippocampal subfield volumes of these participants. There were no significant differences in cognitive functions, and differences in hippocampal subfield volume changes were found between women and men. Moreover, no significant differences between the participants scanned using scanner 1 and scanner 2 were identified with regard to percentage changes in cognitive performances and hippocampal subfield volumes (Supplementary Table S3).

We further explored the relationships between percentage changes in cognitive measures and hippocampal subfield volume changes in the CA4 and molecular layer regions in the longitudinal sample. However, there was no significant correlation between

Table 2
Regression models assessing the relationship of education with the adjusted volumes (mm3) of the hippocampal subfields in the cross-sectional sample

Hippocampal subfields	Models for the entire sample[a]				Models for women[b]			Adjusted R² (p-value)	Models for men[b]			Adjusted R² (p-value)
	Beta	t	p	Adjusted R² (p-value)	Beta	t	p		Beta	t	p	
CA1	0.082	0.979	0.329	0.130 (0.000)	0.016	0.143	0.887	0.039 (0.103)	0.188	1.405	0.166	0.115 (0.022)
CA2/3	0.129	1.525	0.130	0.110 (0.000)	0.035	0.302	0.764	0.039 (0.101)	0.263	2.023	0.048[c]	0.164 (0.005)
CA4	0.117	1.395	0.165	0.133 (0.000)	0.039	0.337	0.737	0.035 (0.117)	0.239	1.866	0.068	0.186 (0.003)
GC-DG	0.121	1.464	0.146	0.150 (0.000)	0.045	0.394	0.695	0.040 (0.098)	0.245	1.899	0.063	0.174 (0.004)
Fimbria	0.098	1.164	0.247	0.123 (0.000)	−0.015	−0.131	0.896	−0.009 (0.519)	0.244	1.853	0.069	0.140 (0.011)
Subiculum	0.075	0.897	0.372	0.127 (0.000)	0.023	0.196	0.845	0.023 (0.180)	0.167	1.269	0.210	0.140 (0.011)
Presubiculum	0.053	0.618	0.538	0.094 (0.001)	0.001	0.005	0.996	0.016 (0.230)	0.144	1.070	0.290	0.098 (0.035)
Parasubiculum	−0.039	−0.439	0.661	0.028 (0.096)	0.034	0.294	0.770	0.023 (0.184)	−0.124	−0.884	0.381	0.020 (0.257)
Molecular layer	0.107	1.306	0.194	0.167 (0.000)	0.034	0.302	0.763	0.059 (0.048)	0.234	1.810	0.076	0.173 (0.004)
HATA	0.045	0.544	0.587	0.151 (0.000)	0.004	0.033	0.974	0.044 (0.084)	0.133	1.051	0.298	0.202 (0.002)
Hippocampal tail	0.047	0.559	0.577	0.124 (0.000)	−0.049	−0.439	0.662	0.106 (0.007)	0.204	1.514	0.136	0.103 (0.031)
Hippocampal fissure	0.030	0.334	0.739	−0.013 (0.716)	0.064	0.544	0.588	0.002 (0.377)	−0.009	−0.062	0.951	0.018 (0.271)
Whole hippocampus	0.094	1.145	0.254	0.168 (0.000)	0.012	0.109	0.914	0.063 (0.040)	0.236	1.868	0.067	0.205 (0.001)

Key: CA, cornu ammonis; CMMSE, Chinese version of the Mini-Mental State Examination; GC-DG, granule cell layer of the dentate gyrus; HATA, hippocampus-amygdala transition area.
  [a] Models include age, sex, education, and CMMSE score as covariates.
  [b] Models include age, education, and CMMSE score as covariates.
  [c] p < 0.05.

RBANS domain index change score and hippocampal subfield volume change in the entire sample, women, and men.

3.4. Associations between education and percentage changes in the hippocampal subfield volumes

We further examined the relationship of education with the percentage change in the volume of CA2/3 region in the longitudinal sample. After controlling for age, sex, CMMSE score, and scanner, the regression model reflected that a higher education level was associated with a lower atrophy in the CA2/3 region (Beta = -0.322, $p = 0.046$).

When the linear regression analysis was stratified by sex, the relationship of education with hippocampal subfield volume changes differed between the 2 sexes. Among men, a higher education level was marginally associated with a lower percentage change in the volume of the CA2/3 region (Beta = -0.439, $p = 0.060$) at a significant level. In women, association between education and percentage change in the volume of the CA2/3 region was not significant (Beta = 0.035, $p = 0.892$).

4. Discussion

The present study provides new evidence regarding the relationship of education with hippocampal subfield volume in healthy older adults. There were 2 major findings. First, our cross-sectional analysis revealed significant positive correlation between the education level and the volume of CA2/3 in men but not in women. This finding suggests that the lifespan development of these hippocampal subfields in men may be associated with the education level. Second, our longitudinal analysis examining the change in this hippocampal subfield during a 15-month period demonstrated that a higher education level was associated with less atrophy in the CA2/3 region in men. This finding not only validates the cross-sectional results but also demonstrates that the association of education with the age-related changes in CA2/3 can be observed even over a short interval of 15 months. These cross-validated results of our study indicate that education selectively slows the atrophy of the CA2/3 subfield in men. Our findings from both the cross-sectional and longitudinal studies support the relationship of education with the progression of normal aging in the hippocampus and a few of its subfields, in addition to supporting the presence of sex differences in the effects of education.

Compared to previous studies, which investigated the relationship of education with the volume of the entire hippocampus, an advantage of the present study is that we mapped the relationship of education with the hippocampal subfields. The CA3—4/DG volume has been linked to associative memory, pattern separation, and pattern completion performance (Rolls, 2013). Another study revealed that the CA2 region plays a critical role in the formation of social memory and that it is uniquely altered during age-related dementia (Chevaleyre and Piskorowski, 2016). Combined with prior knowledge, our findings suggest that education may be associated with the age-related decline in memory performance. However, our results suggest that hippocampal subfield volumes are related to cognitive function only in cross-sectional analysis but not in longitudinal analysis.

Investigating the changes in hippocampal subfields seems to be a more plausible approach in developmental studies, as demonstrated in the present study. The absence of association between the education level and the entire hippocampal volume shown in the present study is consistent with the results of prior studies (Liu et al., 2012; Shpanskaya et al., 2014). A study by Liu et al. (2012) noted no relationship between years of schooling and hippocampal volume after controlling for age, sex, intracranial volume, MMSE

score, and country of origin in healthy older adults. Similarly, Shpanskaya et al. (2014) identified no effects of education on the hippocampal volume in cognitively healthy participants; however, they observed a significant association between higher education level and larger hippocampal volume in AD. They speculated that education is likely to have a direct effect on hippocampal volume in AD. In the present study, education level was marginally associated with the entire hippocampal volume at a significant level in men but not in women. It is possible that pooling men and women in the previous analyses diluted the finding. However, Liu et al. (2012) and Shpanskaya et al. (2014) did not stratify their analyses by sex; therefore, no further comparison is possible. In contrast, the present study indicates that the relationship of education with a few hippocampal subfields is obvious. These results explain the negative findings of the studies evaluating the entire hippocampal volume and underscore the need for more thorough investigations focusing on hippocampal subfields in studies examining the relationship between education and the hippocampus.

The sex difference we observed in the present study is well supported by a recent report showing sex differences in the structure, function, and plasticity of the CA3 pyramidal cells in rodents (Scharfman and MacLusky, 2017). Studies in healthy human participants have similarly demonstrated sex differences in the hippocampal structure (Crivello et al., 2014 ; ; Krogsrud et al., 2014). For instance, several reports indicated that women have larger hippocampal volumes and hippocampal subfield volumes than men (Neufang et al., 2009; Szabo et al., 2003), as reflected in this study. Conversely, pooling men and women in the same sample may yield a null result, as revealed in our study and in a recent study by Kang et al. (2018). As noted above, pooling could have led to the null result for both sexes and stratification could have led to a positive association in men in the present study. However, Kang et al. (2018) did not stratify their analyses by sex, so it is unclear what role pooling may have played in that study. Unfortunately, many other studies have not stratified analyses by sex; therefore, there is little to compare the present results with. One exception is Crivello et al. (2014) reporting greater hippocampal atrophy over time in women than in men. The non-cognitively impaired cohort in this study is much older (65—82 years) than the unimpaired cohort studied here (65—75 years), which allows explanations such as increased Alzheimer lesions in women. For now, the large difference between men and women in the present study remains unexplained, but it is likely interesting and important. We speculate that there may be 2 explanations. First, there may be sex differences in the aging processes of the hippocampus and its subfields, with men and women of the same age developing different associations between hippocampal structure and their early experience. Second, education experience may shape neural circuits differently for different sexes. A large-scale lifespan developmental study is required to provide evidence for these speculations.

With regard to the implications of this study for cognitive reserve (CR), our findings imply that education is an important CR avenue; however, studies clarifying the specific effects of education on different target populations are needed. Education is the most frequently used proxy of CR (Farfel et al., 2013). According to the CR hypothesis (Opdebeeck et al., 2016; Stern, 2006, 2009), education has a direct neuroprotective effect on brain structure or pathology, and some portion of education-related CR in humans is localized to the hippocampus (Shpanskaya et al., 2014). In most previous studies, the hippocampus as a whole was employed to study the influence of education on the hippocampal volume, and sex differences in the hippocampal structures were ignored. Education may affect cognitive abilities differently. This is supported by a recent study showing that education can specifically modulate

several cognitive domains, including working memory, some executive functions, and visuoconstructive abilities (Roldán-Tapia et al., 2017).

In addition, brain reserve hypothesis posits that a larger brain volume contributes to the preservation of cognitive function (Sumowski et al., 2013). Our result revealing that the volume of eTIV was significantly smaller in women than in men is consistent with a previous report (Passe et al., 1997). However, no relationship between cognitive function and eTIV was observed in the present study. We failed to detect any association between education and eTIV. Brain structural parameters, such as total brain volume, are reportedly influenced by genetic and environmental factors (Batouli et al., 2014). However, education is only one of the important environmental factors, which may play an important role in defining volume changes in the memory-related brain regions. Thus, our results support that education was associated with the volume of CA2/3 but not with the volume of the entire brain.

The present findings should be interpreted while keeping a few limitations in mind. First, even though we adjusted the analyses for some major confounders (such as age, sex, and eTIV), other factors (e.g., occupational attainment, leisure, and cognitive activities) might also have modulated cognitive function and brain structure (Arenaza-Urquijo et al., 2015), thereby affecting the associations. Second, in our cross-sectional analysis, the association between education level and hippocampal subfield volumes could not achieve significance after a false discovery rate correction for multiple comparisons; this was due to limited sample size and potentially small effect size in the cross-sectional data. However, we could consider this cross-sectional analysis as a discovery step and verify the findings of this step further in the longitudinal analysis. Combining the results of both the analyses helped reduce the possibility of type I error in our conclusion on the association between education and hippocampal subfield volume change. Future studies combining functional MRI and genomic data are critical in establishing the effects of education, as a major CR approach, on different populations and cognitive components.

5. Conclusions

In summary, our cross-sectional and longitudinal analyses demonstrate that education selectively slows down the atrophy of the hippocampal subfield CA2/3 in men. These findings emphasize the need for additional extensive developmental studies evaluating the influence of environmental factors on hippocampal subfield changes and elucidating the effects that education, as a major CR approach, has on different populations and cognitive components.

Disclosure

The authors have no actual or potential conflicts of interest.

Acknowledgements

All study participants provided informed consent, and the study design was approved by the Human Research Ethics Committees at the participating institutions.

All authors have reviewed the contents of the manuscript being submitted, approve of its contents, and validate the accuracy of the data.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2019.03.019.

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