Age Differences in Hippocampal Subfield Volumes from Childhood to Late Adulthood

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ABSTRACT: The hippocampus is composed of distinct subfields: the four cornu ammonis areas (CA1-CA4), dentate gyrus (DG), and subiculum. The few *in vivo* studies of human hippocampal subfields suggest that the extent of age differences in volume varies across subfields during healthy childhood development and aging. However, the associations between age and subfield volumes across the entire lifespan are unknown. Here, we used a high-resolution imaging technique and manually measured hippocampal subfield and entorhinal cortex volumes in a healthy lifespan sample ($N = 202$), ages 8–82 yrs. The magnitude of age differences in volume varied among the regions. Combined CA1-2 volume evidenced a negative linear association with age. In contrast, the associations between age and volumes of CA3-DG and the entorhinal cortex were negative in mid-childhood and attenuated in later adulthood. Volume of the subiculum was unrelated to age. The different magnitudes and patterns of age differences in subfield volumes may reflect dynamic microstructural factors and have implications for cognitive functions across the lifespan. © 2015 Wiley Periodicals, Inc.

KEY WORDS: development; aging; CA1; dentate gyrus; entorhinal cortex

INTRODUCTION

The hippocampus has long been a focus of research of normal and pathological development in many mammalian species (see Small et al., 2011 for a review). Study of hippocampal integrity and function in humans typically focuses on samples representing discrete age groups (e.g., children, see Ofen, 2012 for a review; or adults, see Raz and Kennedy, 2009 for a review). Although there are lifespan studies of developmental differences in the cerebral cortex (Sowell et al., 2003), little is known of the developmental trajectory of the hippocampal formation across the entire lifespan. Longitudinal investigations of children and adolescents suggest that total hippocampal volume stabilizes in children as early as the age of 4 yrs (Gogtay et al., 2006; Sullivan et al., 2011; Mattai et al., 2011). Studies of healthy adults reveal consistent, monotonic decline in total hippocampal volume that begins in early adulthood and possibly accelerates toward the seventh or eighth decades (Raz et al., 2005, 2010). The hippocampus, however, is not a uniform structure and its components or subfields differ dramatically in their cytoarchitectonic, vascular, and electrophysiological properties (Duvernoy, 1988; Jones and McHugh, 2011; Lavenex and Lavenex, 2013), and thus may follow different developmental trajectories across the lifespan.

The hippocampal subfields include four compartments of Cornu ammonis (CA1-CA4), the dentate gyrus, and the subiculum complex that includes in addition to subiculum proper, pre- and parasubiculum. The subfields are structurally similar across mammalian species (Amaral and Lavenex, 2006), and knowledge of rodent and primate neuroanatomy has provided insights into the anatomy of the human hippocampal formation. Notably, the subfields appear to follow different trajectories in development (see Jones and McHugh, 2011; Lavenex and Lavenex, 2013 for reviews) and in aging (see Keuker et al., 2002 for a review).

Recent developments in high-resolution magnetic resonance imaging (MRI) as well as advancement in manual (e.g., Mueller et al., 2007; Mueller and Weiner, 2009; Malykhin et al., 2010; Shing et al., 2011; Bender et al., 2013; Raz et al., 2014) and computerized (La Joie et al., 2010; Kerchner et al., 2014; Wisse et al., 2014; Yushkevich et al., 2014) segmentation have allowed studying human hippocampal subfields *in vivo*. Yet, the bulk of the literature focuses on adults, with only three studies devoted to neuroanatomy of hippocampal subfields in healthy children, and none addressed age differences across the lifespan.

A handful of extant studies of hippocampal subfield development produced discrepant findings. Larger volumes of CA1 and dentate gyrus were observed in older children in two samples (Krogsrud et al., 2014; Lee et al., 2014), whereas similar age differences in the subiculum were found in one study (Krogsrud et al., 2014) but not in the other (Lee et al., 2014). A single longitudinal study of children and young adults (8–21 yrs of age) revealed significant shrinkage of all hippocampal regions over a period of 2.5 yrs (Tamnes et al., 2014). Although as a rule, the results of longitudinal studies that allow evaluating true trajectories of developmental change should be preferred to

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inferences based on cross-sectional design (e.g., Lindenberger et al., 2011), the comparison of the extant developmental studies of hippocampal subfields is undermined by methodological limitations. The most significant is the limited validity of semi-automated methods of hippocampal subfield parcellation, such as FreeSurfer (de Flores et al., 2014), and the results may be particularly biased because of significant differences in reliability and validity coefficients across the subfields. The problem may be exacerbated when the measurements are performed on images of lower resolution than the one for which the methods were developed, as is the case for the studies by Tamnes et al. (2014) and Krogsrud et al. (2014).

Extant studies of healthy aging have been solely cross-sectional comparisons and have largely agreed upon a pattern of age effects that differentiate across hippocampal subfields. For example, several of the studies have reported age-invariance of subiculum volume (Mueller et al., 2007; Mueller and Weiner, 2009; Shing et al., 2011; Bender et al., 2013; Raz et al., 2014, but see La Joie et al., 2010). In healthy older adults, combined CA1-2 volume is negatively related to age, from young adulthood into the eighth decade of life (Mueller et al., 2007; Mueller and Weiner 2009; Shing et al., 2011; Bender et al., 2013; Wisse et al., 2014). At least in adults, CA1-2 seems more vulnerable than the other subfields to vascular risk factors (Shing et al., 2011; Bender et al., 2013) and genetic variations that are associated with vascular risk and Alzheimer's disease (Mueller and Weiner, 2009; Kerchner et al., 2014; but see Mueller et al., 2008; Raz et al., 2014). The evidence for adult age differences in the dentate gyrus has been less consistent: some studies report significant negative associations with age (Mueller et al., 2007; Mueller and Weiner, 2009; Pereira et al., 2014; Wisse et al., 2014), while others do not (Shing et al., 2011; Bender et al., 2013; Raz et al., 2014).

Although comparing extant studies can provide some hint of the lifespan trajectory of each subfield, such comparisons are hindered by variability in measurement methods and statistical analyses across studies of children, adolescents, and younger and older adults. Thus, an examination of age differences in the volume of hippocampal subfields in a cohesive sample of children and adults drawn from the same population, scanned in the same MRI scanner and measured with the same methods would improve our understaning of lifespan age differences in the components of the hippocampal formation.

This study was designed to examine age-related differences in hippocampal subfield volumes in a broad age range covering the lifespan from childhood to late adulthood and to address some of the shortcomings outlined above. We employed a high-resolution imaging protocol that allows for reliable manual demarcation of the hippocampal subfields; a procedure that has gained standing within the literature and has provided convergent results of adult age differences across laboratories that sampled different populations of participants and used different MRI scanners (Mueller et al., 2007; Mueller and Weiner, 2009; Shing et al., 2011; Bender et al., 2013; Raz et al., 2014). We applied this method to a large cross-sectional sample of healthy participants who ranged in age 8–82 yrs. On the basis of the extant evidence, we hypothesized that the subfields would demonstrate differential associations with age across the lifespan. Combined CA1-2 volume was expected to show a non-linear negative association with age, with smaller volumes associated only with advanced age. The combined CA3-dentate gyrus volume was expected to show a negative association with age beginning in childhood. No age differences were expected in the subiculum volume across the lifespan. In addition to the hippocampal subfields, we measured the entorhinal cortex, a structure that has multiple connections with several hippocampal subfields. Because longitudinal evidence suggests that the entorhinal cortex develops early and is stable by age 8 yrs (Gogtay et al., 2004) and decreases modestly in adulthood (Raz et al., 2005), we expected to find a nonlinear pattern of age-related differences in entorhinal cortex volume across the lifespan.

MATERIALS AND METHODS

Participants

Participants were recruited from the Metro Detroit, MI area as part of ongoing longitudinal studies of neural correlates of memory development in children (ages 8–25 yrs; 32% of the sample) and of the cognitive and neural correlates of aging (ages 18–82 yrs; 68% of the sample). The lifespan sample consisted of 202 individuals (64% female; 77% Caucasian), ages 8–82 yrs ($M = 38.58$, $SD = 20.68$). See Table 1 for a description of sample demographics and Figure 1 for a frequency distribution of the sample by age. The adult sample overlaps 48% with our previous publication (Raz et al., 2014).

All participants met criteria for normal development and healthy aging. Participants reported right-hand dominance, spoke English as the first language, and were screened for neurological and psychiatric disease, learning disorders, and head trauma. In addition, adults were screened for cardiovascular and endocrine diseases, diabetes, cancer, dementia (Mini-Mental State Exam ≥ 26; Folstein et al., 1975) and depression (Center for Epidemiological Studies Depression questionnaire (CES-D) ≤ 16; Radloff, 1977). Adult participants were normotensive, based upon absence of a medical diagnosis, or observed resting blood pressure measurements below clinical criteria (140 mm Hg systolic and 90 mm Hg diastolic). Blood pressure was the average of four measurements using a mercury sphygmomanometer (BMS 12-S25) with a standard blood pressure cuff (Omron Professional) on the left arm while the participant was seated and resting the forearm on a table. Not included in the sample of 202 participants, an additional 57 adults were excluded: 39 were hypertensive, 5 scored above the cut-off on the CES-D, one had an incomplete MRI dataset, and 12 were scanned but excluded for motion artifacts that interfered with reliable measurement; one child was also excluded for poor quality images. Severe motion artifacts and compromised image contrast that interfered with manual tracing were determined qualitatively on a case basis by the agreement of two expert

TABLE 1.

Sample Descriptors

	Children (8–17 yrs)	Adults (18–49 yrs)	Older adults (50–82 yrs)	Total sample
N	37	91	74	202
% Female	46%	63%	76%	64%
Age (years)	12.78 ± 3.13	29.96 ± 9.99	62.07 ± 7.74	38.58 ± 20.68
Education (years)	8.19 ± 3.06	15.79 ± 1.77	16.26 ± 2.31	14.57 ± 3.77
Systolic pressure (mm Hg)		112.85 ± 8.40	120.60 ± 9.71	117.10 ± 9.90
Diastolic pressure (mm Hg)		72.28 ± 6.05	73.94 ± 5.61	73.19 ± 5.85

Note: Sample means ± standard deviations are shown. Blood pressure was measured only in adults.

raters that the anatomical details of the high-resolution scan were not adequately visualized.

Image Acquisition

For the lifespan sample, structural imaging was acquired on a 3T Siemens Verio (Siemens Medical AG, Erlangen, Germany) full-body magnet at the same imaging site. The high-resolution proton density-weighted turbo spin echo (PD-TSE) sequence for hippocampal subfields was acquired with the same parameters for the lifespan sample: voxel size = 0.4 mm × 0.4 mm × 2.0 mm (30 slices); echo time (TE) = 17 ms; repetition time (TR) = 7,150 ms; flip angle = 120°; pixel bandwidth = 96 Hz/pixel; turbo factor 11; field of view (FOV) = 280 × 512 mm^2. A portion of the sample (32%, ages 8–25 yrs) was scanned with a 32-channel head coil, whereas for 68% of the sample (ages 18–82 yrs), the images were acquired with a 12-channel head coil. The two head coils used in this study had similar signal-to-noise ratio (SNR) in the high-resolution hippocampal subfield volumetry PD-TSE scans: $t = -0.58$, $P = 0.57$ ($N = 15$ brains per coil, 32-channel coil mean SNR = 22.84, SD = 4.20; 12-channel coil mean SNR = 22.05, SD = 3.27).

In addition, T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequences were acquired for intracranial volume correction. For the majority of the sample (68%, ages 18–82 yrs) the images were acquired with the following parameters: TR = 1,680 ms; TE = 3.51 ms; inversion time (TI) = 900 ms; flip angle = 9.0°; pixel bandwidth = 180 Hz/pixel; generalized autocalibrating partially parallel acquisitions (GRAPPA) acceleration factor PE = 2; voxel size 0.67 mm × 0.67 mm × 1.34 mm. The remainder of the sample (32%, ages 8–25 yrs) was scanned with the following parameters: TR = 2,200 ms; TE = 4.26 ms; TI = 1,200 ms; flip angle = 9.0°; pixel bandwidth = 130 Hz/pixel; GRAPPA acceleration factor PE = 2; interpolated voxel size 0.5 mm × 0.5 mm × 1.0 mm.

Manual Demarcation of the Regions of Interest

Hippocampal subfields and entorhinal cortex (EC) were manually demarcated following previously reported rules (Bender et al., 2013) that were adapted from Shing et al. (2011; as modified from Mueller et al., 2007; Mueller and Weiner, 2009); see Figure 2 for an example of manual tracings. Two expert raters (A.M.D. and A.R.B.) manually demarcated regional boundaries with a stylus on a 21-in. digitizing tablet (Wacom Cintiq) using Analyze v10.0 software (Mayo Clinic, Rochester, MN). Image intensities were inverted to mimic a T1-weighted image that was more familiar to the operators. Rater reliability was confirmed by an intraclass correlation coefficient with an assumption of random raters [formula ICC(2); Shrout and Fleiss, 1979] on a sub-sample of 12 cases that was representative of the sample age range and included both males and females. The standard of reliability was at least 0.85 for measures in separate hemispheres and 0.90 for total volume of each region (see Table 2). Only after meeting this standard of reliability, the two expert operators expeditiously traced each case, with randomized assignment of first and second rater. For each case, the first rater would trace the ranged slices and the second rater would review and revise the tracings. In this way, possible rater bias was mitigated and randomly distributed. For a detailed description of training and tracing procedures employed in our laboratory see Raz et al. (2004).

Regions of interest (ROIs) included subiculum, CA1-2 combined in a single region, and CA3-4 and the dentate gyrus also combined into a single region (CA3-DG). Hemispheric ranges were allowed to differ by starting slice based on lateral

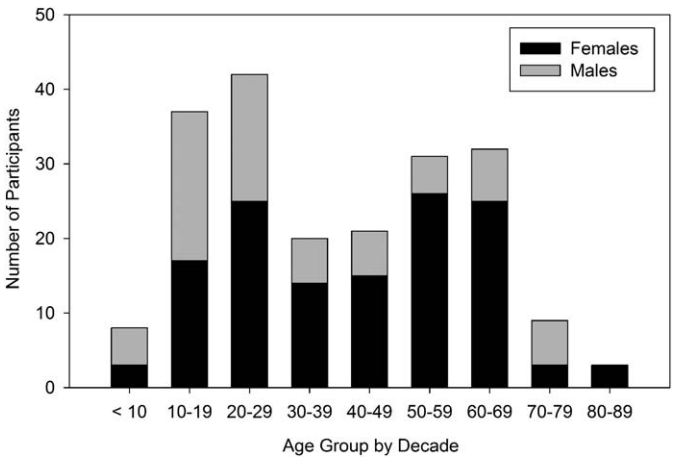

FIGURE 1. A distribution of the number of participants represented by decade age-bin across the lifespan. The color shading of each bar represents the distribution of each sex: black—female; gray—male.

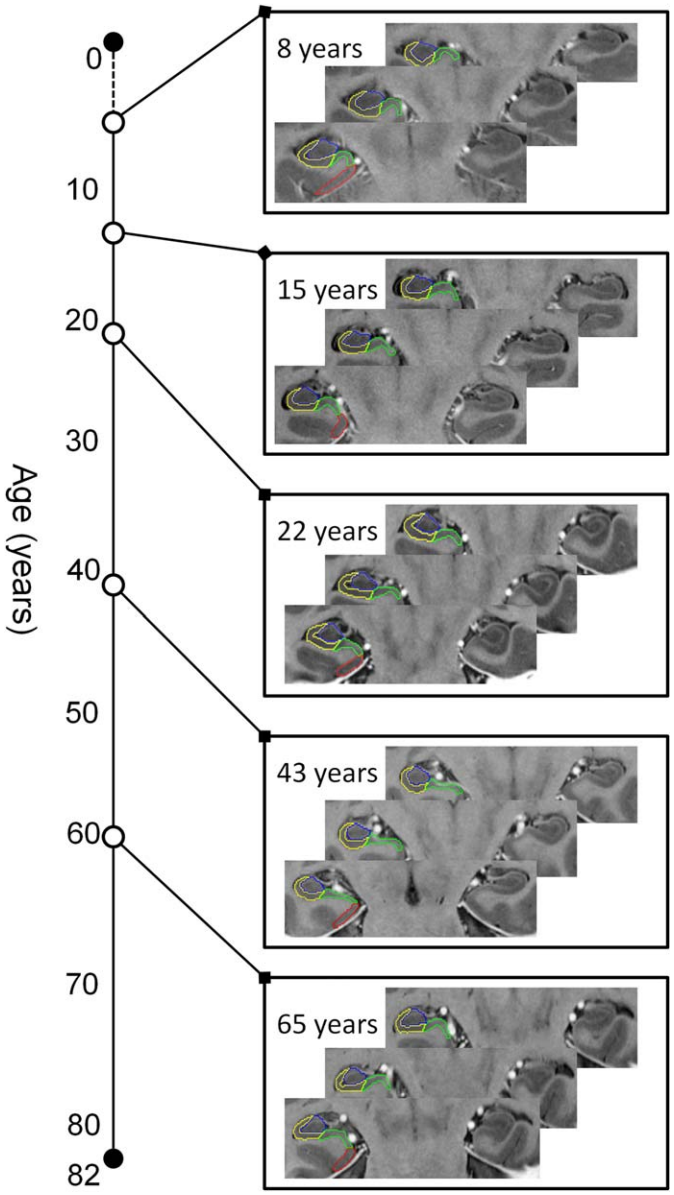

FIGURE 2. Hippocampal subfields and entorhinal cortex: representative images from individuals sampled across the lifespan (ages 8–82 yrs). Within each individual data set, the three images are contiguous slices (0.4 × 0.4 in-plane resolution and 2-mm slice thickness) and represent the range sampled for hippocampal subfield volumetry. Image intensity gray scale is inverted to accommodate demarcation. Red—entorhinal cortex; green—subiculum; yellow—CA1-2; blue—CA3-DG. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

anatomical differences. The hippocampal subfield range began with the slice on which the head of the hippocampus was no longer visible (posterior to the uncal sulcus). All hippocampal regions were traced on three contiguous slices of the anterior hippocampus body. EC was traced on six contiguous slices, extending five slices anterior to the start of the subfield range. In spite of possible age differences in hippocampal form (Gogtay et al., 2006) and length (Insausti et al., 2010), the anatomical landmarks that denote the head (see Poppenk et al., 2013) are present in children and adults alike. Therefore, approximately the same anatomical portion of the hippocampal body was measured in each individual.

Volumes of hippocampal subfields and EC were adjusted for intracranial volume via analysis of covariance (Jack et al., 1989). Head size increases with age in normal childhood development (Sgouros et al., 1999), and age was correlated with intracranial volume in the present sample in ages 8–18 yrs ($r = 0.33$, $P = 0.04$). However, the relation between intracranial volume and hemispheric measures of each ROI did not differ between child, adolescent, and adult age groups (all $F(1,196) \leq 1.48$, $P \geq 0.23$); and the slopes were homogeneous between the sexes (all $F(1,196) \leq 0.93$, $P \geq 0.34$). Therefore, correction for intracranial volume via analysis of covariance was applied similarly to the entire sample. The analysis of covariance approach (Jack et al., 1989) minimizes the bias against detection of age differences whilst allowing for appropriate testing of sex differences in subcortical volumes. Without correcting for individual differences in intracranial volume, sexual dimorphism of absolute head size (Sgouros et al., 1999) would introduce undue bias to the analyses.

Intracranial volume was measured on T1-weighted MPRAGE images using the brain extraction tool (Smith, 2002) in FSL following procedures we have reported before (Bender et al., 2013). Briefly, we modified BET procedures from Keihaninejad et al. (2010), applying standard space masking, the betsurf option, and extra-cranial surface estimation, and inspecting the final output of all cases. Together, the procedure provides a robust estimation of intracranial volume from high quality images. A comparison within an age-homogeneous sub-sample of young adults ($N = 38$, ages 19–24) confirmed that the BET procedure produced similar results independent of differences in imaging parameters or head coil for the T1-weighted MPRAGE ($F(1,35) = 0.11$, $P = 0.75$, controlling for sex differences). For the purpose of volume correction, intracranial volume was divided by 1,000 to avoid scaling artifacts.

Data Conditioning and Statistical Analysis

Prior to analysis, data were examined for skew and univariate outliers were winsorized. Analysis was conducted in a general linear modeling (GLM) framework: a 4 (ROI) × 2

TABLE 2.

Reliability of Manual Volume Measures by Region

Region	Left	Right	Total
Subiculum	0.90	0.94	0.93
CA1-2	0.86	0.93	0.91
CA3-DG	0.88	0.96	0.93
Entorhinal cortex	0.91	0.98	0.99

Note: All reported values are intra-class correlation coefficients assuming random raters, ICC(2) (Shrout and Fleiss, 1979). Agreement was established for two raters on a sub-sample of 12 cases. CA—cornu ammonis; DG—dentate gyrus.

(Hemisphere) repeated measure GLM. Omnibus effects were further investigated with post-hoc GLMs separately by ROI with hemisphere as a two-level variable. Treatment of hemisphere as a two-level repeated measure tested for laterality while accounting for collinearity of the measures and correcting for multiple comparisons. Hemispheric differences were interpreted only when this model variable was significant. All models included as the independent variables age (centered at the sample mean), centered age^2 as a nonlinear component, and sex; bivariate interactions between age and sex were tested and removed from the model if not significant. Nominal significance was set as $P = 0.05$ with a Huynh-Feldt correction, and Bonferroni correction ($\alpha' = 0.01$) was applied for multiple post-hoc comparisons. Further, to avoid spurious results introduced by unequal sample sizes across all ages of the lifespan (see Fig. 1), significant age effects were bootstrapped with bias-correction (5,000 draws, 100% of the observed sample) to produce 95% confidence intervals (CI). Finally, nonlinear age effects that were significant beyond the linear component and survived multiple comparison correction were further examined with polynomial trend analysis to determine the nature of the function. In the event that multiple nonlinear functions fitted the data equally well, the lowest order nonlinear component was accepted.

RESULTS

Hippocampal subfields and EC volumes were entered into a 4 (Region) × 2 (Hemisphere) repeated measure GLM, which revealed significant linear (F (1, 198) = 151.62, $P < 0.001$) and nonlinear (F (1, 198) = 27.25, $P < 0.001$) age differences across all regions. Further, as indicated by age × ROI interactions, the magnitude of the linear (F (3, 594) = 70.36, $P < 0.001$) and nonlinear (F (3, 594) = 12.49, $P < 0.001$) age effects differed between regions. Sex, a covariate in all models, was unrelated to volume differences in any region (across regions: F (1, 198) = 0.04, $P = 0.85$; between regions: F (3, 594) = 0.88, $P = 0.41$).

Significant hemisphere × age interactions indicated hemispheric differences that varied by region for both the linear (F (3, 594) = 14.80, $P < 0.001$) and quadratic (F (3, 594) = 4.20, $P = 0.02$) age effects. Thus, additional analyses by hemisphere were conducted. In post-hoc comparisons, the lateral asymmetry was restricted to the linear age effect within the EC (F (1, 198) = 15.53, $P < 0.001$, $\alpha' = 0.01$)—age differences were larger in the left ($r = -0.58$) as compared to the right hemisphere ($r = -0.47$; Steiger (1980) $Z^* = -2.48$, $P = 0.01$). However, there were no hemispheric differences in the magnitude of the nonlinear age effect within the EC [F (1, 198) = 4.05, $P = 0.05$]. Further, there was no evidence for hemispheric asymmetry in age differences in any other region [all F (1, 198) ≤ 5.28, $P \geq 0.02$, $\alpha' = 0.01$]. Because of the lack of hemispheric differences in hippocampal subfield volumes, all additional analyses only included bilateral total volumes of the hippocampal subfields, as well as of the EC.

Nonlinear Age Differences in the Volumes of the EC and CA3-DG

The analysis of bilateral total volumes across ROIs revealed nonlinear age differences in the volumes of the EC (F (1, 198) = 22.74, $P < 0.001$, $\alpha' = 0.01$; linear component: F [1, 198) = 118.39, $P < 0.001$, $\alpha' = 0.01$] and CA3-DG (F (1, 198) = 8.05, $P < 0.01$, $\alpha' = 0.01$; linear component: F (1, 198) = 105.76, $P < 0.001$, $\alpha' = 0.01$). To avoid spurious results from unequal sample sizes of all ages across the lifespan, the nonlinear slopes of EC and CA3-DG were bootstrapped with bias-correction: EC, $b = 0.08$, bias-corrected bootstrapped 95% CI: 0.05/0.11; CA3-DG, $b = 0.02$, bias-corrected bootstrapped 95% CI: 0.01/0.03; examination of both sets of intervals supports a robust effect. The nonlinear effects of age in the EC and CA3-DG were further examined with polynomial trend analysis. Quadratic functions best described the association of age with the EC (F (2, 199) = 66.24, $P < 0.001$, $\alpha' = 0.01$) and CA3-DG (F (2, 199) = 57.32, $P < 0.001$, $\alpha' = 0.01$) volumes. Thus, the association between age and volumes of EC and CA3-DG was negative and linear beginning in mid-childhood and was attenuated in later adulthood, beginning at ~50 yrs of age (see Figs. 3A,B). In a subsample of 74 middle-aged and older adults (age > 49 yrs) no significant age differences in EC volume were found (F (1, 71) = 0.04, $P = 0.84$) or in CA3-DG (F (1, 71) = 2.41, $P = 0.13$). Linear and quadratic age differences in the EC and CA3-DG were of similar magnitudes across the lifespan: $Z^* = 0.18$, $P = 0.86$ for the linear and $Z^* = 1.25$, $P = 0.21$ for the quadratic.

Linear Age Differences in the Volume of CA1-2

Only a linear age-volume association was identified in CA1-2 (F (1, 198) = 30.27, $P < 0.001$, $\alpha' = 0.01$, $b = -0.70$, bias-corrected bootstrapped 95% CI: −0.96/−0.44)—older participants had smaller CA1-2 volumes as compared to younger counterparts. The non-linear component did not survive correction for multiple comparisons (F (1, 198) = 4.41, $P = 0.04$, $\alpha' = 0.01$, $b = 0.01$, bias-corrected bootstrapped 95% CI: −0.003/0.03). We also compared the magnitudes of volume-age associations across ROIs. The linear association with age was smaller in the CA1-2 region ($r = -0.32$) as compared to that in the EC ($r = -0.57$; $Z^* = 4.02$, $P < 0.001$) and in the CA3-DG ($r = -0.58$; $Z^* = 4.15$, $P < 0.001$).

No Age Differences in the Volume of the Subiculum

We observed no age differences in the volume of the subiculum across the lifespan for either linear (F (1, 198) = 1.92, $P = 0.17$, $b = 0.09$, bias-corrected bootstrapped 95% CI: −0.06/0.23) or nonlinear (F (1, 198) = 1.49, $P = 0.22$, $b = 0.01$, bias-corrected bootstrapped 95% CI: −0.004/0.02) age components. See Figure 3 for plots of ICV-adjusted regional volumes by age. (see the Supporting Information for plots of unadjusted regional volumes by age.)

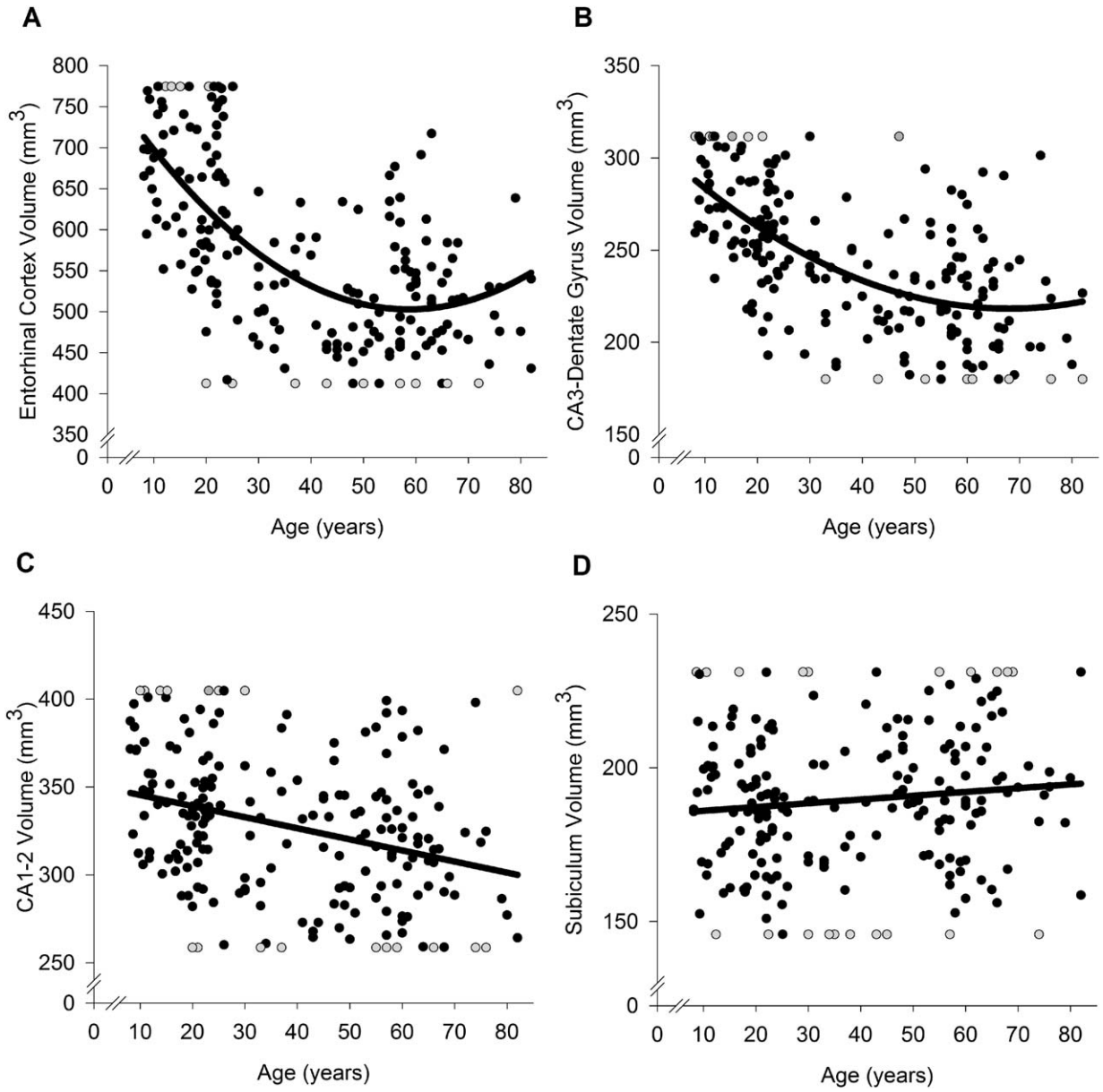

FIGURE 3. The association between volume and age across the lifespan in entorhinal cortex and hippocampal subfields. All volumes are adjusted for intracranial volume (ICV; see text for details). The associations with age in the entorhinal cortex [$Volume_i = 786.06 - 9.65(Age_i) + 0.08(Age_i^2)$] and CA3-dentate gyrus [$Volume_i = 308.02 - 2.64(Age_i) + 0.02(Age_i^2)$] were quadratic (both $p < 0.01$), whereas the effect in CA1-2 was linear [$P < 0.001$; $Volume_i = 351.76 - 0.63(Age_i)$]. There were no age differences in subiculum volume ($P = 0.17$). Gray circles represent data points that were winsorized.

DISCUSSION

In a sample covering almost the entire lifespan, we observed significant age-related differences in hippocampal subfield volumes. The magnitude of these differences and the pattern of the age-volume associations varied across the examined regions. From childhood to late adulthood, the volumes of CA1-2, CA3-DG, and EC but not subiculum showed negative relations with age. The association between younger age and larger CA1-2 volume was linear across the lifespan, whereas nonlinear functions described age differences in volumes of CA3-DG and EC, with associations with age attenuated in later adulthood.

Comparison of the lifespan age differences observed in this study to the results of the investigations based on discrete age groups reveals a mixed pattern of agreement and discrepancy. The observed negative linear associations between age and CA1-2 and CA3-DG volumes in children and young adults are in line with the previously reported longitudinal shrinkage of these regions (Tamnes et al., 2014), but does not replicate

the two cross-sectional findings in a similar age range (Krogsrud et al., 2014; Lee et al., 2014). Smaller CA1-2 in later adulthood as observed here is a common finding in cross-sectional studies of adult aging (Mueller et al., 2007; Mueller and Weiner 2009; Shing et al., 2011; Bender et al., 2013; Wisse et al., 2014). The attenuated later-life age differences in CA3-DG volume are in accord with the studies based on the same or similar populations (Shing et al., 2011; Bender et al., 2013; Raz et al., 2014). They are at odds, however, with reports of age differences extending beyond the fifth decade of life in other studies (Mueller et al., 2007; Mueller and Weiner, 2009; Pereira et al., 2014; Wisse et al., 2014). The observed lack of age differences in subicular volume of aduls replicates most of the extant reports (Mueller et al., 2007; Mueller and Weiner, 2009; Shing et al., 2011; Bender et al., 2013; Raz et al., 2014, but see La Joie et al., 2010). In contrast, the evidence for regional age differences in that structure during childhood remains equivocal (Krogsrud et al., 2014; Lee et al., 2014; Tamnes et al., 2014). Thus, the presented findings add to the extant literature and enable a unique and important lifespan comparison that provides context for developmental findings in isolated segments of the lifespan.

Gaining insight into neurobiological meaning of the observed age-related differences in hippocampal subfield volumes remains a challenge. The main obstacle to such understanding is dearth of comparative studies linking MRI findings with histological characteristics of the brain. Thus, we can only speculate on the possible mechanisms that produce the observed pattern of age differences in local hippocampal subfield volumes. According to histological estimates, the main contributor to gray matter volume is neuropil (more than 50%), with neuronal bodies accounting for an additional 11% of the total (Kassem et al., 2013). Smaller MRI-derived volume of the cerebral gray matter, including the total hippocampus, are believed to reflect neuronal loss (Bobinski et al., 2000), reduction of neuropil (Qui et al., 2013), and decline in intralaminar myelin content (Courchesne et al., 2000), but relative contributions of specific cellular components to the observed age-related differences are unclear. Moreover, no data are available on cellular correlates of hippocampal subfield volumes estimated from MRI. To complicate the matter, the relative contributions of hippocampal volume determinants may vary across the lifespan—at different stages of life, variations in regional volumes may reflect different neural processes. In younger age, the combination of selective neuronal pruning (see Luo and O'Leary, 2005; Holtmaat and Sboboda, 2009 for reviews) and neuropil changes, with possible local contribution of neurogenesis (Gould, 2007) may affect subfield volumes. Neurogenesis, however, is restricted to the DG and, even in that structure, its extent in humans is largely unknown and its role in hippocampal volume maintenance has never been tested (Ho et al., 2013; Breunig et al., 2007). Local volume changes in late adulthood may reflect mainly loss of neuropil and myelin. Thus, although fluctuating dynamic equilibrium of multiple cellular processes may form a mechanistic foundation of the age differences reported here, including the nonlinear effect observed in the CA3-DG, experimental evidence for such mechanism is lacking.

Adult age-related differences in volume of the hippocampal subfields and EC may be influenced by various factors that are not present in children and younger adults, e.g., vascular risk (Shing et al., 2011; Bender et al., 2013). In this context, it is important to note selective vulnerability of CA1 to hypoperfusion, ischemia (Petito and Pulsinelli, 1984) and cardiovascular risk factors (Shing et al., 2011). It remains unclear whether the nonlinearity in age-volume associations observed for EC and CA3-DG reflects a true developmental phenomenon, reflects selection bias in recruiting older adults with excellent health, or is merely a heteroscedasticity artifact. A prospective study of possible modifiers that are relevant across the lifespan or potentially during critical periods of development is necessary to elucidate this phenomenon. Further studies combining noninvasive neuroimaging of the hippocampal subfields with histology in a suitable animal model are necessary to elucidate the neurobiological foundations of the observed age dependency.

The age-related differences in hippocampal subfield and EC volumes across the lifespan are particularly interesting in the context of individual differences in cognition observed in the same period. Other studies of groups sampled across the lifespan have reported differential cognitive correlates of age-related differences (Shing et al., 2011; Bender et al., 2013) and longitudinal change (Tamnes et al., 2014) in subfield volumetry. Smaller CA1-2, CA3-DG, and EC volumes observed in older children and young adults compared to younger children indicate that volume loss may be adaptive to normal development. This may be especially true of the protracted development of memory functions that rely on medial temporal lobe circuits (see Ofen, 2012 for a review). However, because we did not measure cognitive functions with instruments that could be translated across the child-development and adult-aging subsamples, we can only speculate about the functional relevance of the lifespan differences in hippocampal subfield volumes.

In interpreting the results of this study, we are constrained by other limitations. First, the MR images from which the hippocampal subfields were measured, combined high in-plane resolution with relatively thick slices, and our measurements were restricted to a small number of slices through the anterior body of the hippocampus. Therefore, our description of lifespan differences in hippocampal subfields is limited to that segment of the structure. Our measurements are limited to the body of the hippocampus to allow for reliable measures, which is the premise of validity (Carmines and Zeller, 1979). As the anterior hippocampal geometry is complex and the subfields in posterior portions are poorly visualized on the images, attempts to demarcate and measure these regions have thus far failed to meet a high standard of reliability. Although this method is limited to the hippocampal body, it has been successfully employed across laboratories (Mueller et al., 2007; Shing et al., 2011; Bender et al., 2013) and is a valid and reliable measure of the subfields in that part of the hippocampus. It is common across segmentation protocols to apply the same boundary definitions throughout the length of the body (e.g., Mueller et al.,

2007; Ekstrom et al., 2009; Olsen et al., 2013), but morphometry of the hippocampal formation and age effects therein may vary along the long axis of the structure (Gogtay et al., 2006). Acquisition of relatively thick slices, necessitated by constraints of the present study, precludes investigation of possible individual and age-related differences in morphometry along the long axis of the hippocampus (e.g., Gogtay et al., 2006). Our hope is that with improving resolution, especially in higher-strength magnetic fields (e.g., 7T), and extending the range of measurements, future studies may endeavor to explore such differences.

Second, a cross-sectional design is incapable of providing valid estimates of age-related change and individual differences therein (Lindenberger et al., 2011) and constrains the inferential scope of this study. Further, participants at the young and old extremes of the age range are under-represented in this sample. Disproportionate representation of individuals at either end of the lifespan may introduce a bias to the cross-sectional comparisons reported here. Because this lifespan sample was drawn from ongoing longitudinal studies, we intend to address these limitations in future reports, and by including similar cognitive instruments, we intend to investigate the functional relevance of variability in hippocampal subfield volume across the lifespan.

CONCLUSION

The magnitude of age differences in the volume of hippocampal subfields and entorhinal cortex varies across the lifespan. Older age was associated with smaller volume in all examined regions except one. The negative association between volumes of the entorhinal cortex and CA3-DG with age was nonlinear, whereas the age-related differences in CA1-2 volumes were best described by a negative linear slope function. In contrast, subiculum volume evidenced no age-related differences across the lifespan. Differential associations between age and regional volume of these medial temporal lobe structures across the lifespan may reflect different neuronal mechanisms and may have implications for differences in memory performance. The goal of the ongoing longitudinal investigations of lifespan dynamics of hippocampal subfield volumes is to shed light on such structure–function relations.

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