Title: Longitudinal trajectories of hippocampal volume in middle to older age community dwelling individuals

Running Head: Trajectories of Hippocampal Change

Authors:

Mark A Fraser BSc (Hons) ^{a*}

Erin I. Walsh PhD ^{a, b}

Marnie E. Shaw ^c

Walter P. Abhayaratna MBBS, PhD ^d

Kaarin J. Anstey ^{a, e, f}

Perminder S. Sachdev MD, PhD ^g

Nicolas Cherbuin PhD ^a

Affiliations:

^a Centre for Research on Ageing, Health and Wellbeing, Australian National University, Canberra, Australia

^b Population Health Exchange, Research School of Population Health, Australian National University, Canberra, Australia

^c ANU College of Engineering & Computer Science, Australian National University, Canberra, Australia

^d College of Health & Medicine, Australian National University, Canberra, Australia

^e UNSW, Ageing Futures Institute, University of New South Wales, Sydney, Australia

^f Neuroscience Research Australia, Sydney, New South Wales, Australia.

^g Centre for Healthy Brain Ageing (CHeBA), School of Psychiatry, University of New South Wales, Sydney, Australia

***Corresponding author:**

Centre for Research on Ageing, Health and Wellbeing, Florey Building 54, Mills Road, Australian National University, Canberra, ACT 2601, Australia. Tel: +61-2-61256338; Email: mark.fraser@anu.edu.au

Declarations of Interest: None

Title: 101 characters

Running head: 34 characters

Word count: 4302

Abstract: 167

Figures: 2

Tables: 2

Supplementary material: yes

Funding Source

The study was supported by an Australian Government Research Training Program (RTP) Scholarship, NHMRC grant No. 973302, 179805,350833 157125, and ARC grant No. 130101705 and NICTA. Kaarin Anstey is funded by NHMRC Fellowship No. 1102694. This study is NOT industry sponsored

Contributions – CrediT author statement

Mr Fraser: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization

Dr Walsh: Conceptualization, Methodology, Data Curation, Writing - Review & Editing

Dr Shaw: Conceptualization, Data Curation, Writing - Review & Editing

Prof. Abhayaratna: Conceptualization, Resources, Writing - Review & Editing, Funding acquisition

Prof. Anstey: Conceptualization, Resources, Writing - Review & Editing, Funding acquisition

Prof. Sachdev: Conceptualization, Resources, Writing - Review & Editing, Funding acquisition

Prof. Cherbun: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition

Highlights

- Hippocampal volumes followed a non-linear trajectory from middle age
- Volumes decreased on average by 0.18%/yr. in middle age and 0.3%/yr. in older age
- Men demonstrated greater atrophy than women in middle age, but not older age
- Left hippocampus was smaller with greater atrophy compared to the right
- Two-thirds of older age participants maintained stable volumes across the follow-up

Abstract

Understanding heterogeneity in brain ageing trajectories is important to estimate the extent to which ageing outcomes can be optimised. While brain changes in late life are well-characterised, brain changes in middle age are not well understood. In this study we investigated hippocampal change in a generally healthy community-living population of middle (n=421, mean age 47.2 years) and older age (n=411, mean age 63.0 years) individuals, over a follow-up of up to 12 years. Manually traced hippocampal volumes were analysed using multilevel models and latent class analysis to investigate longitudinal ageing trajectories and laterality and sex effects, and to identify subgroups that follow different ageing trajectories. Hippocampal volumes decreased on average by 0.18%/year in middle age and 0.3%/year in older age. Men tended to experience steeper declines than women in middle age only. Three subgroups of individuals following different trajectories were identified in middle age and two in older age. Contrary to expectations, the subgroup containing two-thirds of older age participants maintained stable hippocampal volumes across the follow-up.

Key words: 1. Hippocampus, 2. Aging, 3. Trajectory, 4. Longitudinal, 5. Middle age 6. MRI

1. Introduction

It is important to understand brain changes that can be attributed to the typical ageing process across the lifespan to guide prevention and population health. Yet, neuroimaging research tends to focus on clinically relevant neuropathology (e.g. Alzheimer's disease, Parkinson's disease) in older adults or psychopathology (e.g. schizophrenia, depression) in the young. As a result, the precise dynamics of age related brain changes across the adult lifespan are not well understood, particularly those taking place in middle age.

The hippocampus is a bilateral brain structure that provides a sensitive and practical measure of brain change over time. The hippocampus plays an important role in memory and mood regulation with many factors shown to affect hippocampal structure and function including genetic vulnerabilities (Li et al., 2016), modifiable lifestyle factors (Cherbuin et al., 2015; Durazzo et al., 2013; Erickson et al., 2011), environmental exposure (Hanson et al., 2011), and neuropathology (Braak et al., 1996). Typical hippocampal volume change across the adult lifespan is thought to exhibit a non-linear trajectory that follows a weakly declining slope until around 50 years of age when the trajectory trends downward at an accelerating rate (Coupé et al., 2017; Fjell et al., 2013; Fraser et al., 2015). This trajectory has been demonstrated by a number of comprehensive cross-sectional studies investigating age-related change in brain structure across the adult lifespan (Coupé et al., 2017; Potvin et al., 2016). However, large longitudinal studies investigating the trajectories of age-related hippocampal volume change have tended to focus on groups older than 55 years of age (Armstrong et al., 2019; Cespedes et al., 2017; Leong et al., 2017; Schuff et al., 2012), and the few longitudinal studies that have covered both middle and older age groups tend to have relatively small sample sizes (Narvacan et al., 2017; Pfefferbaum and Sullivan, 2015; Raz et al., 2010; Raz et al., 2005; Taylor et al., 2014). Thus, although the typical hippocampal ageing trajectory across the adult lifespan has been extensively characterised from cross

sectional research, the paucity of longitudinal evidence in middle age represents a gap in the evidence base.

The contribution of several individual characteristics on neurodegeneration trajectories are also insufficiently understood. Hemispheric asymmetry, where the right hippocampus is larger than the left hippocampus by as much as 5.8% in individuals free of neurological disorder (Shi et al., 2009), combined with asymmetric atrophy rates (Ardekani et al., 2019), suggest possible differences in the trajectories of left and right hippocampal volumes. Moreover, although when no adjustment for head size is applied, men demonstrate larger hippocampal volumes than women (Coupé et al., 2017; Goodro et al., 2012; Nobis et al., 2019; Potvin et al., 2016), after controlling for head size this difference often becomes non-significant or reversed (Coupé et al., 2017; Goodro et al., 2012; Narvacan et al., 2017; Nobis et al., 2019; Shen et al., 2019; Taylor et al., 2014). In addition, some longitudinal studies have found no sex differences in atrophy rates (Narvacan et al., 2017; Raz et al., 2010; Taylor et al., 2014), whereas others found higher hippocampal atrophy in elderly women aged 74.7 years (Shen et al., 2019), or higher atrophy in men over 50 years (Armstrong et al., 2019; Nobis et al., 2019). Individual characteristics and varying exposure to risk factors may also interact with sex and individual behaviours to differentially impact hippocampal trajectories (Head et al., 2012; Li et al., 2016; Morra et al., 2009; Shen et al., 2019).

The typical ageing trajectory involving an overall population level decline in hippocampal volumes assumes a homogenous population. However, evidence of differences in cognitive ageing related to education and ethnicity (Zelinski and Jacobucci, 2017), multiple trajectories of cognitive change in pre-clinical Alzheimer's disease (Small and Bäckman, 2007), and multiple trajectories of terminal wellbeing decline (Burns et al., 2014) suggest the possibility of heterogenous ageing trajectories. We hypothesised that there could be multiple subpopulation trajectories of hippocampal ageing that may suggest new combinations of risk factors for future investigation.

To address these questions, we used multilevel models (MLM) and multilevel latent class analysis to investigate longitudinal hippocampal volume ageing trajectories in middle and older age community-living individuals, over a follow-up of up to 12 years. The study aims were to determine the typical hippocampal volume ageing trajectory across middle age to older age, to explore hemispheric and sex trajectory differences, and to identify subgroups of individuals following heterogeneous ageing trajectories and to investigate their characteristics.

2. Materials and methods

2.1 Participants

Study participants were from the Personality and Total Health Through Life (PATH) project, a large longitudinal study investigating ageing, health, cognition, and individual characteristics across the adult lifespan described in detail elsewhere (Anstey et al., 2012; Cherbuin et al., 2009; Maller et al., 2007). Briefly, prospective participants were randomly selected from the electoral rolls of the adjacent cities of Canberra and Queanbeyan, Australia, and as enrolment to vote is compulsory in Australia, this approach provides cohorts that are representative of the population. At baseline, the project surveyed 2404 young adults (YA) aged 20-24 years, 2530 middle aged adults (MA) aged 40-44 years and 2551 older age adults (OA) aged 60-64 years, representing participation rates (PR) of 58.6%, 64.6% and 58.3% of those invited. A randomly selected subsample of the two older cohorts were invited to undergo MRI scans. At wave 1, 478 OA participants (PR = 76.8%) underwent a baseline MRI scan and at the second wave of data collection 431 MA participants (PR = 85.7%) received their baseline MRI scan. MRI sub-study participants were invited to have further scans at each subsequent wave of data collection (Supplementary Figure S1). Participants with epilepsy, stroke, Parkinson's disease, mild cognitive impairment (MCI) or dementia at any wave were excluded from the current study, leaving 421 MA participants and 411 OA participants with a baseline scan. The study sample did not differ from the overall PATH cohort in age, BMI, sex, hypertension, diabetes, or *APOE-ε4* genotype at the time of the baseline scan. However, the study OA participants had higher education levels (0.40 years, 95% CI 0.12, 0.68) and fewer smokers (-4%, 95% CI 0.01, 0.07) compared to OA participants in the overall PATH cohort (Supplementary Table S1). All participants provided written informed consent. The Human Research Ethics Committee of The Australian National University approved the study protocol.

2.2 MRI scan acquisition and pre-processing

T1-weighted three-dimensional structural MRI scans were acquired for all participants. Detailed parameters and methods have been extensively reported elsewhere (Shaw et al., 2017) and are reported in more detail in the supplementary material. In brief, for the first two waves, all participants were imaged with a 1.5 Tesla Phillips Gyroscan ACS-NT scanner (Phillips Medical systems, Best, The Netherlands) for T1-weighted 3D structural MRI using a fast-field echo (FFE) sequence. Due to scanner changes all participants were scanned with a Siemens Avanto scanner (Siemens Medical solutions) at wave 3, and with a Siemens Espree scanner (Siemens Medical solutions) at wave 4. Intensity normalisation and B1 inhomogeneity correction (Sled et al., 1998) were applied on all images using the MINC imaging toolbox (MINC; <http://en.wikibooks.org/wiki/MINC>).

2.3 Volumetric measurement.

Manual hippocampal volumes were measured by experienced neuroscientists (CM, JM, MF) tracing the left and right hippocampus on each slice of a T1-weighted scan in coronal orientation using Analyze Software (Brain Imaging Resource, Mayo Clinic, Rochester, MI, USA). The protocol used for the hippocampal volume measurement has been described in detail elsewhere (Maller et al., 2006; Watson et al., 1997; Watson et al., 1992). Wave 3 and wave 4 scans were resized to 1.0mm³ voxels prior to tracing. Mean reliability measured by intra-class correlation coefficient (ICC2, Shrout and Fleiss, 1979), measured on 10 randomly selected retraced scans, was ≥ 0.88 across all waves (Fraser et al., 2018; Maller et al., 2007; Maller et al., 2006). The hippocampal volumes were normalized to adjust for differences in total intracranial volume (ICV) using the residual method (adjusted volume = raw volume $- \beta \times [\text{ICV} - \text{mean ICV}]$; where β is the slope of the regression of the ROI volume on the ICV) with the estimated total intracranial volume for each participant calculated from the FreeSurfer 5.3 cross-sectional pipeline (Buckner et al., 2004; Pintzka et al., 2015; Raz et al., 2004; Sanfilipo et al., 2004). Total hippocampal volume was calculated as the sum of left and right hippocampal volume for each

participant. Scanner related variance in hippocampal volumes was estimated and removed via an established and published orthogonalisation method (Shaw et al., 2016b). This process was carried out prior to statistical analysis and on the entire PATH data set of MRI scans corresponding to participants aged 44–78 years old, for the purpose of minimising the correlation between the scanner-covariate and participant age. The distributions of hippocampal volumes for each time point and age group were then examined and univariate outliers ($Z > 3.29$) were excluded (Tabachnick et al., 2007).

2.4 Participant characteristics

Age, sex, education, diabetes, and smoking were measured by self-report. Body mass index (BMI) was calculated as $\text{weight (kg)} / [\text{height (m)}]^2$. Blood pressure was objectively measured. Participants with blood pressure above 140mmHg systolic or 90mmHg diastolic (average of two seated measures), or who reported taking blood pressure medication were classified as “hypertensive”. *APOE-ε4* genotype was assessed using DNA collected by cheek swab at wave 1 (Cherbuin et al., 2011). Only OA participants were assessed using the Mini Mental State Examination (MMSE) as this test is not designed to provide cognitive data on middle age adults.

2.5 Statistical analysis

Statistical analysis was performed using R version 3.5.1 (R Core Team, 2018). Multilevel models, using lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages, were used to examine longitudinal changes in hippocampal volumes over time. Significance of fixed effects were assessed with 95% confidence intervals, random effects significance was assessed using χ^2 tests, and Pseudo R^2 was determined using the approach suggested by Nakagawa and Schielzeth (2013). While multilevel models analyse group and individual trajectories, and are tolerant of missing observations and differences in the timing of longitudinal observations, multilevel models assume that all participants are drawn from a single population with common parameters. Therefore, we used the lmm package

(Proust-Lima et al., 2017) to identify subpopulations (latent classes) with ageing trajectories that differ from the overall group trajectory. Significance of sample characteristic differences was assessed using t-tests, χ^2 tests, or Wilcoxon signed-rank tests. Missing hippocampal data (no more than one missing occasion per participant), representing 1.37% of total measured volume were singly imputed using the R Amelia-II package (Honaker et al., 2011).

2.5.1 Hippocampal volume trajectories

Trajectories of hippocampal volume change were investigated using MLMs with the trajectories for the MA and OA cohorts combined investigated first. A linear model with total hippocampal volume as the outcome variable and age, centred at the sample minimum age (44 years), as the predictor was compared using ANOVA to a quadratic model that included a quadratic age term. Next, the effects of hemisphere and sex were investigated. A fully specified linear model with age, hemisphere and sex predictors including two and three-way interactions was compared to a model that included quadratic age predictors (age, age², hemisphere, sex) with two and three-way interactions. Backward deletion using the lmerTest step function (Kuznetsova et al., 2017) was applied to each model before comparison. Model fit of the optimal linear and quadratic models, measured by Akaike information criterion (AIC: Akaike, 1974), was compared using ANOVA with optimal models selected based on model fit and ease of interpretation where model fit was very similar. The process was repeated for the MA cohort with age centred at the sample minimum age (44 years) and then for the OA cohort with age centred at the sample minimum age (60 years).

2.5.2 Latent class analysis

The presence of latent classes (subgroups) was investigated using LCMMs separately for each hemisphere of the MA and OA cohorts (Proust-Lima et al., 2017). LCMMs were modelled using a linear function with age as the predictor. The results were analysed, and the best fitting latent class sets

were selected based on model fit (AIC), stable allocation of individuals to the classes ($>50\%$ of members of each class with posterior probability >0.7), and at least 20 individuals in each class.

The best fitting latent class sets for the left hippocampus and right hippocampus in each cohort were examined for class differences. In each latent class set containing multiple classes, the participant characteristics, measured at baseline, of the smaller classes in each set were compared to the participant characteristics of largest class.

2.5.3 Post hoc analysis

The modelled trajectories were projected back to 20 years of age to test consistency with previous literature that investigated hippocampal volumes in other age groups including young adults. This was achieved by centring age at 80 years and running models backwards in time with the lme4 simulate function used to simulate hippocampal volumes for a cohort of young adults based on the MA data points (Bates et al., 2015). The projected volumes and rates of atrophy were then compared to the results of previous studies that included participants from across the adult lifespan.

3. Results

Baseline Participant characteristics are shown in **Table 1**. Both cohorts had high levels of education with more education in the MA cohort. As expected, the OA cohort had more medical comorbidity and smaller hippocampi at baseline. The intra-class correlations (ICC) indicated a moderate proportion of variance between individuals (MA = 52-55%, OA = 40-44%, All = 47-49%) and this level of between subject variability was suitable for using random individual intercepts. However, insufficient variability in slope was detected across time and therefore random slopes were excluded from the models.

For the combined cohorts, mean hippocampal volume change was -17.3 [95% CI -20.7,-13.9] mm³/year (-0.25%/year) for the total hippocampus, -11.7 [95% CI -13.5,-9.8] mm³/year (-0.34%/year) for the left, and -5.8 [95% CI -7.6,-3.9] mm³/year (-0.17%/year) for the right. For the MA cohort, hippocampal volume change was -12.2 [95% CI -20.3,-4.1] mm³/year (-0.18%/year) for the total hippocampus, -12.6 [95% CI -17.1,-8.0] mm³/year (-0.36%/year) for the left, and 0.5 [95% CI -3.9, 4.9] mm³/year (< 0.1%/year) for the right. For the OA cohort, hippocampal volume change was -21.3 [95% CI -28.2,-14.5] mm³/year (-0.30%/year) for the total hippocampus, -18.7 [95% CI -22.2,-15.1] mm³/year (-0.51%/year) for the left, and -2.7 [95% CI -6.5, 1.2] mm³/year (< -0.1%/year) for the right.

Table 1 Participant characteristics at baseline

	All	Middle Age	Older Age	Middle vs Older Age
Sample, No.	832	421	411	
Age, years (SD)	55.02 (8.05)	47.20 (1.38)	63.03 (1.43)	< 0.001 ^a
Age, years range	44.66 - 65.98	44.66 - 49.80	60.32 - 65.98	
Male, No. (%)	402 (48.32)	189 (44.89)	213 (51.82)	0.045 ^b
Years of Education, (SD)	14.51 (2.42)	14.82 (2.24)	14.19 (2.57)	< 0.001 ^a
BMI, kg/m ² (SD)	26.75 (4.57)	27.07 (4.82)	26.43 (4.28)	0.051 ^a
Hypertension, No. (%)	362 (43.51)	115 (27.32)	247 (60.10)	< 0.001 ^b
Diabetes, No. (%)	42 (5.05)	10 (2.38)	32 (7.79)	< 0.001 ^b
<i>APOE-ε4</i> carrier, No. (%)	215 (25.84)	118 (28.03)	97 (23.60)	0.145 ^b
Smoker, No. (%)	95 (11.42)	66 (15.68)	29 (7.06)	< 0.001 ^b
Hippocampus volume				
Left, mm ³ (SD)	3287 (367)	3345 (330)	3226 (395)	< 0.001 ^a
Right, mm ³ (SD)	3350 (367)	3426 (307)	3269 (407)	< 0.001 ^a
Total, mm ³ (SD)	6638 (692)	6771 (590)	6494 (763)	< 0.001 ^a

Note: BMI = body mass index; a = t-test; b = χ^2 test.

3.1 Hippocampal volume trajectories

The total hippocampal volume trajectory was non-linear for both the combined and OA cohorts. (**Table S2, Models S1-S3, and Figure S2**). When the effects of hemisphere and sex were included, hippocampal volume followed a non-linear trajectory for the combined and MA cohorts, with a linear trajectory for the OA cohort (**Table 2, Models 1-3, and Figure 1**). MA men had significantly larger hippocampal volume and greater rates of volume change compared to women. Furthermore, the right hippocampus was significantly larger compared to the left hippocampus and the rate of volume loss

was significantly lower for the right hippocampus when compared to the left. In OA, the rate of volume loss was significantly lower for the right hippocampus when compared to the left.

Figure 1 Hippocampal volume trajectories in middle and older age. The black line is the average of the trajectories, light blue is the male right hippocampus, orange is the female right hippocampus, dark blue is the male left hippocampus, and red is the female left hippocampus. The grey shadows around the trajectories represents the 95% confidence intervals. The individual data points for females are represented by circles and males by triangles. Longitudinal datapoints for each participant are connected by lines.

Table 2 Trajectory models

<i>Predictors</i>	ALL HCV (Model 1)			MA HCV (Model 2)			OA HCV (Model 3)		
	<i>Estimates</i>	<i>95% CI</i>	<i>p</i>	<i>Estimates</i>	<i>95% CI</i>	<i>p</i>	<i>Estimates</i>	<i>95% CI</i>	<i>p</i>
(Intercept)	3223.75	3166.91 – 3280.60	<0.001	3271.15	3202.25 – 3340.05	<0.001	3275.04	3234.62 – 3315.46	<0.001
Age	5.63	-1.16 – 12.42	0.104	0.99	-17.30 – 19.28	0.915	-18.27	-21.81 – -14.73	<0.001
Age ²	-0.40	-0.59 – -0.20	<0.001	-0.50	-1.69 – 0.68	0.406			
Right side	177.63	124.67 – 230.59	<0.001	73.57	30.62 – 116.51	0.001	20.85	-23.98 – 65.68	0.362
Male	190.46	110.02 – 270.91	<0.001	265.17	169.78 – 360.56	<0.001			
Age × Right	-7.24	-14.12 – -0.37	0.039	10.90	5.31 – 16.50	<0.001	15.32	10.57 – 20.07	<0.001
Age × Male	-15.85	-24.38 – -7.32	<0.001	-59.82	-85.81 – -33.83	<0.001			
Right × Male	-66.14	-126.55 – -5.73	0.032						
Age ² × Male	0.31	0.08 – 0.54	0.008	3.31	1.59 – 5.03	<0.001			
Age ² × Right	0.25	0.05 – 0.45	0.016						
Age × Right × Male	3.66	0.44 – 6.89	0.026						
Random Effects									
σ^2	66282.42			54818.19			74091.46		
τ_{00}	63653.00	Individual		65949.94	Individual		62038.99	Individual	
ICC	0.49			0.55			0.46		
N	825			419			406		
Observations	4222			1958			2264		

Marginal R ² / Conditional R ²	0.095 / 0.538	0.064 / 0.5750	0.063 / 0.490
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Note: MA = Middle age cohort; OA = Older age cohort; All = Both cohorts combined; HCV = Hippocampal volume; σ^2 = Residual random variance; τ_{00} = Individual intercept random variance; ICC = Intra-class correlation between individuals; Model 1 shows impact of age, sex and hemisphere on hippocampal volume; Model 2 shows impact of age, sex and hemisphere on MA hippocampal volume; Model 3 shows impact of age and hemisphere on OA hippocampal volumes.

3.2 Latent class analysis

We estimated up to five class models for each structure and examined the model fit, stability and class sizes to determine whether the optimal class sets had been identified. We did not estimate six or more class models as it was clear that the optimal class sets involved 1-3 classes. Goodness of fit characteristics of the latent class sets are shown in **Supplementary Table S3**, the latent class trajectories are shown in **Figure 2**, and latent class comparisons are shown in **Supplementary Table S4**.

MA left hippocampus: A three class model produced the best fit with groups predominantly differentiated by their baseline volume (low, medium, high). The largest class, Class 1 ($n = 287$) representing 68.5% of the population, had an intercept of 3413.42 [95% CI 3356.01, 3470.83] mm^3 and volume change of -11.05 [95% CI -17.03, -5.07] mm^3/year (-0.32%/year), the next largest class (Class 2, $n = 100$, 23.9%) had a smaller intercept of 3052.97 [95% CI 2971.87, 3134.07] mm^3 , volume change of -16.49 [95% CI -25.53, -7.45] mm^3/year (-0.54%/year), and the smallest class (Class 3, $n = 32$, 7.6%) had the largest intercept of 3931.80 [95% CI 3775.59, 4088.01] mm^3 , and volume change of -14.12 [95% CI -30.17, 1.93] mm^3/year (-0.36%/year). There were no significant differences between Class 1 and Class 2 at baseline. Compared to Class 1, Class 3 was older by 0.82 [95% CI 0.25, 1.38] years, had a 26% [95% CI 7, 45] greater proportion of males.

MA right hippocampus: A two class model produced the best fit with groups differentiated by baseline volume (low, high) and slope of volume change. The largest class (Class 1, $n = 216$, 51.6%) had an intercept of 3536.38 [95% CI 3432.93, 3639.83] mm^3 and volume change of 7.08 [95% CI -0.27, 14.43] mm^3/year (0.20%/year), and the smaller class (Class 2, $n = 203$, 48.4%) had a smaller intercept of 3284.28 [95% CI 3196.02, 3372.54] mm^3 and volume change of -6.98 [95% CI -15.35, 1.39] mm^3/year (-0.21%/year). When the two classes were compared at baseline, there were no significant differences.

OA left hippocampus: A two class model produced the best fit with groups differentiated by both baseline volume and slope (high-declining, low-stable). The largest class (Class 1, $n = 258$, 63.5%) had an intercept of 3097.64 [95% CI 3048.64, 3146.64] mm^3 , and volume change of -2.18 [95% CI -12.16, 7.80] mm^3/year (-0.07%/year) and the smaller class (Class 2, $n = 148$, 36.5%) had an intercept of 3534.36 [95% CI 3389.46, 3679.26] mm^3 , and volume change of -41.43 [95% CI -55.23, -27.63] mm^3/year (-1.17%/year). When the two classes were compared at baseline, there were no significant differences.

OA right hippocampus: A two class model met the criteria in terms of lowest AIC and all class sizes greater than 20 but failed the stability criteria with class 2 having < 50% of members with a posterior probability > 0.7. Thus, the single class model was selected.

3.3 Sensitivity analysis

Although individuals with neurological disorders were not included in this study, latent class analysis was repeated for the OA cohort with low MMSE participants (MMSE < 27; 3.7%) excluded to assess the impact of prodromal neurocognitive disorders. The analysis produced the same latent class structure and characteristics (**Table S5**).

3.4 Post hoc analysis

The trajectory models and observed MA and OA data were used to estimate hippocampal volumes for younger adults from 20 years of age and estimate atrophy rates by decade from 20 to 80 years. The maximum total hippocampal volume of 6866 mm^3 was estimated to occur at 26 years of age, and the model predicted a 10.9% volume loss by age 80 (**Supplementary Figure S3, Table S6**).

Figure 2 Hippocampal trajectories corresponding to different subgroups of individuals identified by latent class analysis. Upper left = MA left hippocampus (Class1 $n = 287$, Class2 $n = 100$, Class 3 $n = 32$), Upper right = MA right hippocampus (Class1 $n = 216$, Class2 $n = 203$), Lower left = OA left hippocampus (Class1 $n = 258$, Class2 $n = 148$), Lower right = OA right hippocampus (Class1 $n = 406$). Class 1: Trajectory is represented by a solid line and the data points by circles, Class 2: Trajectory is represented by short dashed line and data points by triangles, Class 3: Trajectory is represented by long dashed line and data points by squares. Longitudinal datapoints for each participant are connected by lines.

4. Discussion

We investigated longitudinal hippocampal ageing trajectories in samples of middle age and older age adults living in the community. Particularly notable findings were that, while a consistent and progressive decrease in volume was observed across the age range considered, the atrophy rates were lower than would have been predicted based on previous literature (Barnes et al., 2009; Fraser et al., 2015), especially in the older aged cohort. Furthermore, subgroup analysis demonstrated that hippocampal volumes remained stable for most older age adults with volume decline identified in only a third of this population. This novel finding augments the limited existing evidence of heterogeneous ageing outcomes (Burns et al., 2015; Burns et al., 2019; Small and Bäckman, 2007).

4.1 Trajectories

Hippocampal volumes followed a non-linear trajectory across middle age and older age, remaining non-linear when effects of hemisphere and sex were included, consistent with previous cross-sectional (Allen et al., 2005; Coupé et al., 2017; Potvin et al., 2016; Walhovd et al., 2011; Ziegler et al., 2015) and longitudinal research (Fraser et al., 2015; Pfefferbaum and Sullivan, 2015; Schmidt et al., 2018; Schuff et al., 2012). When the older age cohort was examined separately, the trajectory appeared non-linear for the combined hippocampus, but linear when independent hemisphere contributions were included in the model. Left hippocampal volumes were smaller and experienced higher atrophy compared to the right. This suggests that linear hemispheric trajectory differences may appear non-linear when left and right hippocampal volumes are combined. Results from earlier longitudinal studies of total hippocampal volumes using smaller samples and fewer time points produced equivocal results with respect to linearity (Leong et al., 2017; Narvacan et al., 2017; Raz et al., 2010; Schuff et al., 2012). However, the present findings in a large sample with long follow-up demonstrates that while the hippocampal volume trajectories are non-linear across middle and older age, there is significant heterogeneity related to hemisphere and sex.

Middle but not older aged women had a smaller mean normalised left hippocampal volume and a lower atrophy rate compared to men. This finding is consistent with that of a recent large ($n=19,793$) cross sectional study (Nobis et al., 2019; Sudlow et al., 2015) that found that the rate of volume loss is lower in women between 50 and 60 years of age after which men and women have similar mean bilateral hippocampal volumes and follow a similar trajectory. It is possible that the lower rates of atrophy in women are related to the effects of lifestyle factors, or reduced estrogen levels after menopause (Barth et al., 2016; Raz et al., 2004; Wnuk et al., 2012; Zárte et al., 2017), or to greater convergence of risk factors after mid-life (e.g. obesity, sedentary lifestyle). Earlier trajectory studies, that did not separately model the effects of sex or hemisphere, identified an inflection point in hippocampal volume change around 50 years of age (Coupé et al., 2017; Fjell et al., 2013). Thus, it is possible that there may be different inflection points in hippocampal trajectories for men and women, and this question warrants future investigation of sex differences across the third to the seventh decades.

When compared to meta-analysis benchmarks, the estimated total atrophy rates from the current study were lower than Barnes et al. (2009) and within the confidence interval range of a later meta-analysis of 27 studies characterising hippocampal atrophy in normal ageing (Fraser et al., 2015). The relatively low rates of hippocampal atrophy in the current study are consistent with the corresponding rates of age-related cortical thinning from the same cohort samples (Shaw et al., 2016a; Shaw et al., 2016b)

When we estimated hippocampal volumes from 20 to 80 years, the models suggested that volumes peaked in the mid-20s consistent with previous development and ageing studies (Allen et al., 2005; Fjell et al., 2013; Lupien et al., 2007; Uematsu et al., 2012; Wellington et al., 2013), and in agreement with a large longitudinal developmental study in children and young adults aged 5 to 25 years (Fish et al., 2019), but earlier than Coupe et al. (2017). Predicted volumes from the models were consistent with previous literature taking into account differences in volumetry methods used (Allen et al., 2005; Scahill et al., 2003; Uematsu et al., 2012). We projected that total hippocampal volume would have declined

by almost 11% from 20 to 80 years of age, slightly lower than the 13% decline reported by Allen et al. (2005).

4.2 Latent class analysis

We identified subgroups of individuals with distinct hippocampal trajectories. In middle age, the main difference between the subgroups related to baseline hippocampal volume. This indicates that differences in hippocampal volume were larger between people than over time, and can be interpreted as evidence that different subgroups of hippocampal atrophy over time may be difficult to differentiate in middle age.

In older age, we identified two subgroups for the left hippocampus with the largest group having a smaller hippocampus at baseline and stable volume at a life stage when mean volumes are expected to decline. When we looked at the trajectories of the right hippocampus for the two subgroups, we found a similar pattern with right hippocampal volumes declining in the smaller subgroup and not declining in the larger subgroup (**Supplementary Figure S4**). We compared demographic characteristics of the subgroups at baseline, but no significant differences or consistent trends were identified (Livingston et al., 2017). We considered the possibility that sample attrition with healthier/unhealthy participants dropping out in one or the other group, contributed to these findings. While the participants who dropped out from the stable group were seven months older than those who remained in the study, they did not differ in sex ratio, education, BMI, hypertension, diabetes, *APOE-ε4* genotype, or smoking status. For the declining group, apart from somewhat more women than men dropping out, the participants who dropped out were not significantly different to those who remained. Given that there were no significant sex differences in the older cohort trajectories, the small age difference is unlikely to explain the different subgroup trajectories (**Supplementary Table S7**). Similar heterogeneous trajectories have previously been found for MMSE scores and for terminal decline in mental health and wellbeing in older adults (Burns et al., 2015; Small and Bäckman, 2007). This suggests that average

trajectories may not be representative of the majority of the population. The implication here is that previous research may have overestimated the rate of decline for much of population while underestimating decline for a minority. This could be explored by larger studies focusing on detecting subgroups with enough statistical power to identify differences between the stable and declining groups.

4.3 Laterality

We found that left hippocampal volumes were smaller and experienced higher atrophy rates compared to the right. Such rightward hippocampal volumetric asymmetry has been reported from childhood, continues throughout adult life (Giedd et al., 1996; Potvin et al., 2016; Uematsu et al., 2012; Woolard and Heckers, 2012), and is thought to be related to hemispheric functional specialisation (Reuter-Lorenz et al., 2000; Toga and Thompson, 2003). Increased left hippocampal atrophy in normal ageing may be related to the left hippocampus being more vulnerable to cardiovascular factors such as chronic hypoperfusion, increased thickness of the carotid artery, and increased incidence of stroke (Cherbuin et al., 2010; Giannakopoulos et al., 2007). Left hippocampal atrophy in normal ageing appears to be ameliorated by the recruitment of right hemisphere regions resulting in less asymmetric cognitive processing (Reuter-Lorenz et al., 2000). However, rightward volumetric asymmetry decreases in adults experiencing subjective cognitive decline (SCD; Yue et al., 2018) or AD (Shi et al., 2009). In AD, increasing grey matter loss in the right hemisphere coincide with significant cognitive deficits (Giannakopoulos et al., 2007; Thompson et al., 2003). Hence increasing rightward asymmetry appears to be associated with normal ageing, while asymmetry reduction appears to be associated with later-stage AD. In the current study, while the population demonstrates a typical asymmetric normal ageing pattern, the largest subgroup appears to have stable hippocampal volumes with little age related decline suggesting that this group may have a lower risk of developing dementia in later life.

4.4 Strengths and limitations

The main strengths of this study include the large sample size, narrow age cohorts of middle age and older aged community-living adults, longitudinal follow-up of up to 12 years, manual tracing of left and right hippocampal volumes, and precise modelling of both hemisphere and sex. Limitations include possible sample selection biases and attrition over time. The number of sequential scans was limited, with up to three for the middle age and four for the older aged cohort. To our knowledge, this is the largest manually traced longitudinal study of hippocampal ageing in middle age and older age populations investigating both hemisphere and sex.

4.5 Conclusions

In this study we found that hippocampal volumes followed a non-linear trajectory declining in middle age by 0.18% per year increasing to 0.30% per year in older age. Left hippocampal volumes were lower and demonstrated greater atrophy compared to the right. We also found that middle age women had smaller left hippocampal volume and lower rates of atrophy compared to middle age men. However, these sex differences dissipated at older ages, suggesting that there may be different critical ages for hippocampal ageing in men and women. Finally, the population contained a number of subgroups that differed in terms of baseline hippocampal volume and rates of change. Most striking was that the largest older age group demonstrated stable hippocampal volumes across the period of the study suggesting that there is no one-size-fits-all normative trajectory of hippocampal change in ageing.

Acknowledgements:

The authors are grateful to Marc Budge, Tony Jorm, Jerome Maller, Chantal Réglade-Meslin, Peter Butterworth, Simon Easteal, Helen Christensen and the PATH team. Funding: The study was supported by an Australian Government Research Training Program (RTP) Scholarship, NHMRC grant No. 973302, 179805, 350833 157125, and ARC grant No. 130101705 and NICTA. Kaarin

Anstey is funded by NHMRC Fellowship No. 1102694. This research was partly undertaken on the National Computational Infrastructure (NCI) facility in Canberra, Australia, which is supported by the Australian Commonwealth Government. The authors declare no competing financial interests.

References

- Akaike, H., 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19(6), 716-723.
- Allen, J.S., Bruss, J., Brown, C.K., Damasio, H., 2005. Normal neuroanatomical variation due to age: The major lobes and a parcellation of the temporal region. *Neurobiol. Aging* 26(9), 1245-1260.
- Anstey, K.J., Christensen, H., Butterworth, P., Easta, S., Mackinnon, A., Jacomb, T., Maxwell, K., Rodgers, B., Windsor, T., Cherbuin, N., Jorm, A.F., 2012. Cohort profile: the PATH through life project. *International journal of epidemiology* 41(4), 951-960.
- Ardekani, B.A., Hadid, S.A., Blessing, E., Bachman, A.H., 2019. Sexual Dimorphism and Hemispheric Asymmetry of Hippocampal Volumetric Integrity in Normal Aging and Alzheimer Disease. *AJNR. American journal of neuroradiology* 40(2), 276-282.
- Armstrong, N.M., An, Y., Beason-Held, L., Doshi, J., Erus, G., Ferrucci, L., Davatzikos, C., Resnick, S.M., 2019. Sex Differences in Brain Aging and Predictors of Neurodegeneration in Cognitively Healthy Older Adults. *Neurobiol. Aging* 81, 146-156.
- Barnes, J., Bartlett, J.W., van de Pol, L.A., Loy, C.T., Scahill, R.I., Frost, C., Thompson, P., Fox, N.C., 2009. A meta-analysis of hippocampal atrophy rates in Alzheimer's disease. *Neurobiol. Aging* 30(11), 1711-1723.
- Barth, C., Steele, C.J., Mueller, K., Rekkas, V.P., Arélin, K., Pampel, A., Burmann, I., Kratzsch, J., Villringer, A., Sacher, J., 2016. In-vivo Dynamics of the Human Hippocampus across the Menstrual Cycle. *Sci. Rep.* 6(1).
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1).
- Braak, H., Braak, E., Bohl, J., Reintjes, R., 1996. Age, neurofibrillary changes, A beta-amyloid and the onset of Alzheimer's disease. *Neurosci. Lett.* 210(2), 87-90.
- Buckner, R.L., Head, D., Parker, J., Fotenos, A.F., Marcus, D., Morris, J.C., Snyder, A.Z., 2004. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *Neuroimage* 23(2), 724-738.
- Burns, R.A., Byles, J., Magliano, D.J., Mitchell, P., Anstey, K.J., 2015. The utility of estimating population-level trajectories of terminal wellbeing decline within a growth mixture modelling framework. *Social Psychiatry and Psychiatric Epidemiology* 50(3), 479-487.
- Burns, R.A., French, D., Luszcz, M., Kendig, H.L., Anstey, K.J., 2019. Heterogeneity in the Health and Functional Capacity of Adults Aged 85+ as Risk for Mortality. *Journal of the American Geriatrics Society* 67(5), 1036-1042.
- Burns, R.A., Mitchell, P., Shaw, J., Anstey, K.J., 2014. Trajectories of terminal decline in the well-being of older women: the DYNOPTA project. *Psychol. Aging* 29(1), 44-56.
- Cespedes, M.I., Frapp, J., McGree, J.M., Drovandi, C.C., 2017. Comparisons of neurodegeneration over time between healthy ageing and Alzheimer's disease cohorts via Bayesian inference. *BMJ open*.
- Cherbuin, N., Anstey, K.J., Réglade-Meslin, C., Sachdev, P.S., 2009. In Vivo Hippocampal Measurement and Memory: A Comparison of Manual Tracing and Automated Segmentation in a Large Community-Based Sample. *PLoS ONE* 4(4).
- Cherbuin, N., Réglade-Meslin, C., Kumar, R., Sachdev, P., Anstey, K.J., 2010. Mild cognitive disorders are associated with different patterns of brain asymmetry than normal aging: the PATH through life study. *Frontiers in psychiatry* 1.

- Cherbuin, N., Sachdev, P.S., Anstey, K.J., 2011. Mixed handedness is associated with greater age-related decline in volumes of the hippocampus and amygdala: the PATH through life study. *Brain and behavior* 1(2), 125-134.
- Cherbuin, N., Sargent-Cox, K., Fraser, M.A., Sachdev, P., Anstey, K.J., 2015. Being overweight is associated with hippocampal atrophy: the PATH Through Life Study. *International journal of obesity* (2005) 39(10), 1509-1514.
- Coupé, P., Catheline, G., Lanuza, E., Manjón, J.V.V., Initiative, A.s., 2017. Towards a unified analysis of brain maturation and aging across the entire lifespan: A MRI analysis. *Human brain mapping* 38(11), 5501-5518.
- Durazzo, T.C., Meyerhoff, D.J., Nixon, S., 2013. Interactive effects of chronic cigarette smoking and age on hippocampal volumes. *Drug and Alcohol Dependence* 133(2), 704-711.
- Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabo, A., Chaddock, L., Kim, J.S., Heo, S., Alves, H., White, S.M., Wojcicki, T.R., Mailey, E., Vieira, V.J., Martin, S.A., Pence, B.D., Woods, J.A., McAuley, E., Kramer, A.F., 2011. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A* 108(7), 3017-3022.
- Fish, A.M., Nadig, A., Seidlitz, J., Reardon, P.K., Mankiw, C., McDermott, C.L., Blumenthal, J.D., Clasen, L.S., Lalonde, F., Lerch, J.P., Chakravarty, M.M., Shinohara, R.T., Raznahan, A., 2019. Sex-biased trajectories of amygdalo-hippocampal morphology change over human development. *Neuroimage*, 116122.
- Fjell, A., Westlye, L., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Holland, D., Dale, A., Walhovd, K., 2013. Critical ages in the life course of the adult brain: nonlinear subcortical aging. *Neurobiol. Aging* 34(10).
- Fraser, M.A., Shaw, M.E., Anstey, K.J., Cherbuin, N., 2018. Longitudinal Assessment of Hippocampal Atrophy in Midlife and Early Old Age: Contrasting Manual Tracing and Semi-automated Segmentation (FreeSurfer). *Brain Topogr.*, 1-14.
- Fraser, M.A., Shaw, M.E., Cherbuin, N., 2015. A systematic review and meta-analysis of longitudinal hippocampal atrophy in healthy human ageing. *Neuroimage*.
- Giannakopoulos, P., Gold, G., Kövari, E., von Gunten, A., Imhof, A., Bouras, C., Hof, P.R., 2007. Assessing the cognitive impact of Alzheimer disease pathology and vascular burden in the aging brain: the Geneva experience. *Acta neuropathologica* 113(1), 1-12.
- Giedd, J.N., Vaituzis, A.C., Hamburger, S.D., Lange, N., Rajapakse, J.C., Kaysen, D., Vauss, Y.C., Rapoport, J.L., 1996. Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: Ages 4-18 years. *J Comp Neurology* 366(2), 223-230.
- Goodro, M., Sameti, M., Patenaude, B., Fein, G., 2012. Age effect on subcortical structures in healthy adults. *Psychiatry Research: Neuroimaging* 203(1), 38-45.
- Hanson, J.L., Chandra, A., Wolfe, B.L., Pollak, S.D., 2011. Association between Income and the Hippocampus. *PLoS One* 6(5).
- Head, D., Bugg, J.M., Goate, A.M., of ..., F.-A.M., 2012. Exercise engagement as a moderator of the effects of APOE genotype on amyloid deposition. *Archives of*
- Honaker, J., King, G., Blackwell, M., 2011. Amelia II: A program for missing data. *Journal of Statistical Software* 45(7), 1-47.
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* 82(13).
- Leong, R., Lo, J.C., Sim, S., Zheng, H., Tandi, J., Zhou, J., Chee, M., 2017. Longitudinal brain structure and cognitive changes over 8 years in an East Asian cohort. *Neuroimage* 147, 852-860.
- Li, B., Shi, J., Gutman, B.A., Baxter, L.C., Thompson, P.M., Caselli, R.J., Wang, Y., Initiative, A.s., 2016. Influence of APOE Genotype on Hippocampal Atrophy over Time - An N=1925 Surface-Based ADNI Study. *PLoS One* 11(4).

- Livingston, G., Sommerlad, A., Orgeta, V., Costafreda, S.G., Huntley, J., Ames, D., Ballard, C., Banerjee, S., Burns, A., Cohen-Mansfield, J., Cooper, C., Fox, N., Gitlin, L.N., Howard, R., Kales, H.C., Larson, E.B., Ritchie, K., Rockwood, K., Sampson, E.L., Samus, Q., Schneider, L.S., Selbæk, G., Teri, L., Mukadam, N., 2017. Dementia prevention, intervention, and care. *The Lancet* 390(10113), 2673-2734.
- Lupien, S., Evans, A., Lord, C., Miles, J., Pruessner, M., Pike, B., Pruessner, J., 2007. Hippocampal volume is as variable in young as in older adults: Implications for the notion of hippocampal atrophy in humans. *NeuroImage* 34(2).
- Maller, J.J., Anstey, K.J., Réglade-Meslin, C., Christensen, H., Wen, W., Sachdev, P., 2007. Hippocampus and amygdala volumes in a random community-based sample of 60-64 year olds and their relationship to cognition. *Psychiatry research* 156(3), 185-197.
- Maller, J.J., Réglade-Meslin, C., Anstey, K.J., Sachdev, P., 2006. Sex and symmetry differences in hippocampal volumetrics: before and beyond the opening of the crus of the fornix. *Hippocampus* 16(1), 80-90.
- Morra, J.H., Tu, Z., Apostolova, L.G., Green, A.E., Avedissian, C., Madsen, S.K., Parikshak, N., Toga, A.W., Jack, C.R., Jr., Schuff, N., Weiner, M.W., Thompson, P.M., 2009. Automated mapping of hippocampal atrophy in 1-year repeat MRI data from 490 subjects with Alzheimer's disease, mild cognitive impairment, and elderly controls. *Neuroimage* 45(1 Suppl), S3-15.
- Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4(2), 133-142.
- Narvacan, K., Treit, S., Camicioli, R., Martin, W., Beaulieu, C., 2017. Evolution of deep gray matter volume across the human lifespan. *Human Brain Mapping* 38(8), 3771-3790.
- Nobis, L., Manohar, S.G., Smith, S.M., Alfaro-Almagro, F., Jenkinson, M., Mackay, C.E., Husain, M., 2019. Hippocampal volume across age: Nomograms derived from over 19,700 people in UK Biobank. *NeuroImage: Clinical* 23, 101904.
- Pfefferbaum, A., Sullivan, E.V., 2015. Cross-sectional versus longitudinal estimates of age-related changes in the adult brain: overlaps and discrepancies. *Neurobiol. Aging* 36(9), 2563-2567.
- Pintzka, C.W.S., Hansen, T.I., Evensmoen, H.R., Håberg, A.K., 2015. Marked effects of intracranial volume correction methods on sex differences in neuroanatomical structures: a HUNT MRI study. *Front. Neurosci.* 9, 238.
- Potvin, O., Mouiha, A., Dieumegarde, L., Duchesne, S., Initiative, A.s., 2016. Normative data for subcortical regional volumes over the lifetime of the adult human brain. *Neuroimage*.
- Proust-Lima, C., Philipps, V., Lique, B., 2017. Estimation of Extended Mixed Models Using Latent Classes and Latent Processes: The R Package lcmm. *Journal of Statistical Software* 78(2).
- R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raz, N., Ghisletta, P., Rodrigue, K.M., Kennedy, K.M., Lindenberger, U., 2010. Trajectories of brain aging in middle-aged and older adults: regional and individual differences. *Neuroimage* 51(2), 501-511.
- Raz, N., Gunning-Dixon, F., Head, D., Rodrigue, K.M., Williamson, A., Acker, J.D., 2004. Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume. *Neurobiol. Aging* 25(3), 377-396.
- Raz, N., Lindenberger, U., Rodrigue, K.M., Kennedy, K.M., Head, D., Williamson, A., Dahle, C., Gerstorf, D., Acker, J.D., 2005. Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb Cortex* 15(11), 1676-1689.
- Reuter-Lorenz, P.A., Jonides, J., Smith, E.E., Hartley, A., Miller, A., Marshuetz, C., Koeppel, R.A., 2000. Age Differences in the Frontal Lateralization of Verbal and Spatial Working Memory Revealed by PET. *Journal of Cognitive Neuroscience* 12(1), 174-187.
- Sanfilipo, M.P., Benedict, R.H.B., Zivadinov, R., Bakshi, R., 2004. Correction for intracranial volume in analysis of whole brain atrophy in multiple sclerosis: the proportion vs. residual method. *Neuroimage* 22(4), 1732-1743.

- Scahill, R.I., Frost, C., Jenkins, R., Whitwell, J.L., Rossor, M.N., Fox, N.C., 2003. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Archives of Neurology* 60(7), 989-994.
- Schmidt, M.F., Storrs, J.M., Freeman, K.B., Jack, C.R., Turner, S.T., Griswold, M.E., Mosley, T.H., 2018. A comparison of manual tracing and FreeSurfer for estimating hippocampal volume over the adult lifespan. *Human Brain Mapping*.
- Schuff, N., Tosun, D., Insel, P., Chiang, G., Truran, D., Aisen, P., Jr, C., Weiner, M., 2012. Nonlinear time course of brain volume loss in cognitively normal and impaired elders. *Neurobiol. Aging* 33(5).
- Shaw, M.E., Abhayaratna, W.P., Sachdev, P.S., Anstey, K.J., Cherbuin, N., 2016a. Cortical Thinning at Midlife: The PATH Through Life Study. *Brain Topogr.* 29(6), 875-884.
- Shaw, M.E., Nettersheim, J., Sachdev, P.S., Anstey, K.J., Cherbuin, N., 2017. Higher Fasting Plasma Glucose is Associated with Increased Cortical Thinning Over 12 Years: The PATH Through Life Study. *Brain Topogr.* 30(3), 408-416.
- Shaw, M.E., Sachdev, P.S., Anstey, K.J., Cherbuin, N., 2016b. Age-related cortical thinning in cognitively healthy individuals in their 60s: the PATH Through Life study. *Neurobiol. Aging* 39, 202-209.
- Shen, S., Zhou, W., Chen, X., Zhang, J., for Initiative, A.s., 2019. Sex differences in the association of APOE ϵ 4 genotype with longitudinal hippocampal atrophy in cognitively normal older people. *European Journal of Neurology*.
- Shi, F., Liu, B., Zhou, Y., Yu, C., Jiang, T., 2009. Hippocampal volume and asymmetry in mild cognitive impairment and Alzheimer's disease: Meta-analyses of MRI studies. *Hippocampus* 19(11), 1055-1064.
- Shrout, P.E., Fleiss, J.L., 1979. Intraclass correlations: uses in assessing rater reliability. *Psychological bulletin* 86(2), 420.
- Sled, J.G., Zijdenbos, A.P., Evans, A.C., 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE transactions on medical imaging* 17(1), 87-97.
- Small, B.J., Bäckman, L., 2007. Longitudinal Trajectories of Cognitive Change in Preclinical Alzheimer's Disease: A Growth Mixture Modeling Analysis. *Cortex* 43(7), 826-834.
- Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M., Liu, B., Matthews, P., Ong, G., Pell, J., Silman, A., Young, A., Sprosen, T., Peakman, T., Collins, R., 2015. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLOS Medicine* 12(3).
- Tabachnick, B.G., Fidell, L.S., Ullman, J.B., 2007. Using multivariate statistics. Pearson Boston, MA.
- Taylor, J.L., Scanlon, B.K., Farrell, M., Hernandez, B., Adamson, M.M., Ashford, W.J., Noda, A., Murphy, G.M., Weiner, M.W., 2014. APOE-epsilon4 and aging of medial temporal lobe gray matter in healthy adults older than 50 years. *Neurobiol. Aging* 35(11), 2479-2485.
- Thompson, P.M., Hayashi, K.M., De Zubicaray, G., Janke, A.L., Rose, S.E., Semple, J., Herman, D., Hong, M.S., Dittmer, S.S., Doddrell, D.M., 2003. Dynamics of gray matter loss in Alzheimer's disease. *The Journal of Neuroscience* 23(3), 994-1005.
- Toga, A.W., Thompson, P.M., 2003. Mapping brain asymmetry. *Nature Reviews Neuroscience* 4(1), 37-48.
- Uematsu, A., Matsui, M., Tanaka, C., Takahashi, T., Noguchi, K., Suzuki, M., Nishijo, H., 2012. Developmental trajectories of amygdala and hippocampus from infancy to early adulthood in healthy individuals. *PLoS One* 7(10).
- Walhovd, K.B., Westlye, L.T., Amlie, I., Espeseth, T., Reinvang, I., Raz, N., Agartz, I., Salat, D.H., Greve, D.N., Fischl, B., Dale, A.M., Fjell, A.M., 2011. Consistent neuroanatomical age-related volume differences across multiple samples. *Neurobiol. Aging* 32(5), 916-932.
- Watson, C., Jack, C.R., Cendes, F., 1997. Volumetric magnetic resonance imaging. Clinical applications and contributions to the understanding of temporal lobe epilepsy. *Archives of neurology* 54(12), 1521-1531.

- Watson, C.M.D.P., Andermann, F.M.F., Gloor, P.M.D.P., Jones-Gotman, M.P., Peters, T.P., Evans, A.P., Olivier, A.M.D.P.F., Melanson, D.M., Leroux, G.R., 1992. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic resonance imaging. *Neurology* 42(9), 1743-1750.
- Wellington, R.L., Bilder, R.M., Napolitano, B., Szeszko, P.R., 2013. Effects of age on prefrontal subregions and hippocampal volumes in young and middle-aged healthy humans. *Human Brain Mapping* 34(9), 2129-2140.
- Wnuk, A., Korol, D.L., Erickson, K.I., 2012. Estrogens, hormone therapy, and hippocampal volume in postmenopausal women. *Maturitas* 73(3), 186-190.
- Woolard, A.A., Heckers, S., 2012. Anatomical and functional correlates of human hippocampal volume asymmetry. *Psychiatry research* 201(1), 48-53.
- Yue, L., Wang, T., Wang, J., Li, G., Wang, J., Li, X., Li, W., Hu, M., Xiao, S., 2018. Asymmetry of Hippocampus and Amygdala Defect in Subjective Cognitive Decline Among the Community Dwelling Chinese. *Frontiers in psychiatry* 9, 226.
- Zárate, S., Stevnsner, T., Gredilla, R., 2017. Role of Estrogen and Other Sex Hormones in Brain Aging. Neuroprotection and DNA Repair. *Frontiers in Aging Neuroscience* 9, 430.
- Zelinski, E.M., Jacobucci, R., 2017. HETEROGENEITY IN COGNITIVE CHANGE TRAJECTORIES OF OLDER ADULTS OBSERVED FROM STRUCTURAL EQUATION MODEL TREES. *Alzheimer's & Dementia* 13(7), P584-P585.
- Ziegler, G., Penny, W.D., Ridgway, G.R., Ourselin, S., Friston, K.J., Initiative, A.s., 2015. Estimating anatomical trajectories with Bayesian mixed-effects modeling. *Neuroimage* 121, 51-68.

Supplementary Information

Figure S1 Personality and Total Health Through Life (PATH) project and MRI sub-study project data collection waves.

Table S1 Comparison of PATH project samples to study samples at baseline

	PATH Middle Age	Study Middle Age	p	PATH Older Age	Study Older Age	p
Sample, No.	2354	421		2550	411	
Age, years (SD)	47.09 (1.46)	47.20 (1.38)	0.147 ^a	63 (1.47)	63.03 (1.43)	0.674 ^a
Age, years range	44.15 - 50.17	44.66 - 49.80		60.23 - 66.81	60.32 - 65.98	
Male, No. (%)	1103 (46.86)	189 (44.89)	0.457 ^b	1316 (51.61)	213 (51.82)	0.935 ^b
Years of Education, (SD)	14.83 (2.23)	14.82 (2.24)	0.919 ^a	13.78 (2.84)	14.19 (2.57)	0.005^a
BMI, kg/m ² (SD)	26.91 (5.08)	27.07 (4.82)	0.561 ^a	26.76 (4.8)	26.43 (4.28)	0.164 ^a
Hypertension, No. (%)	644 (27.36)	115 (27.32)	0.986 ^b	1600 (62.75)	247 (60.10)	0.304 ^b
Diabetes, No. (%)	74 (3.14)	10 (2.38)	0.397 ^b	193 (7.57)	32 (7.79)	0.877 ^b
<i>APOE</i> -ε4 carrier, No. (%)	598 (25.4)	118 (28.03)	0.257 ^b	641 (25.14)	97 (23.60)	0.504 ^b
Smoker, No. (%)	431 (18.31)	66 (15.68)	0.195 ^b	285 (11.18)	29 (7.06)	0.012^b

Note: BMI = body mass index; MMSE = Mini-mental state examination; a = t-test; b = χ^2 test; c = Wilcoxon signed-rank test.

MRI scan acquisition

T1-weighted three-dimensional structural MRI scans were acquired for all participants. For the first two waves, participants were imaged with a 1.5 Tesla Phillips Gyroscan ACS-NT scanner (Phillips Medical systems, Best, The Netherlands) for T1-weighted 3D structural MRI in coronal orientation using a fast-field echo (FFE) sequence with the following parameters: Wave 1 repetition time (TR) = 28.05ms, echo time (TE) = 2.64ms, flip angle (FA) = 30°, matrix size of 256×256 , field of view (FOV) of 260×260 mm, slice thickness = 2mm, with each contiguous slice overlapping by 1mm yielding over-contiguous 1mm coronal slices with a voxel size of 1.032mm^3 . In wave 2, the FFE sequence for the first 164 participants, all from the 40s cohort, was (TR/TE/FA = 8.84ms/3.55ms/8°) and for all others was (TR/TE/FA = 8.93ms/3.57ms/8°), the matrix size was 256×256 with contiguous slices of 1.5mm, and a voxel size of $1.5 \times 1.0 \times 1.0$ mm. At wave 3, scans were acquired on a Siemens Avanto scanner (Siemens Medical solutions) in sagittal orientation (TR/TE/FA = 1160ms/4.17ms /15°), matrix size 512×512 , contiguous 1.0mm slices giving a voxel size of $1.0 \times 0.5 \times 0.5$ mm. At wave 4, scans were acquired on a Siemens Espreo scanner (Siemens Medical solutions), sagittal orientation (TR/TE/FA = 1160ms/4.24ms/15°), matrix size 512×512 , contiguous 1.0mm slices, and voxel size of $1.0 \times 0.5 \times 0.5$ mm (Shaw et al., 2017). Intensity normalisation and B1 inhomogeneity correction (Sled et al., 1998) were applied on all images using the MINC imaging toolbox (MINC; <http://en.wikibooks.org/wiki/MINC>).

Table S2 Total hippocampus trajectory models

	ALL HCV (Model S1)			MA HCV (Model S2)			OA HCV (Model S3)		
<i>Predictors</i>	<i>Estimates</i>	<i>95% CI</i>	<i>p</i>	<i>Estimates</i>	<i>95% CI</i>	<i>p</i>	<i>Estimates</i>	<i>95% CI</i>	<i>p</i>
(Intercept)	6765.35	6681.69 – 6849.01	<0.001	6776.45	6701.34 – 6851.56	<0.001	6506.15	6444.14 – 6568.16	<0.001
Age	-8.53	-18.84 – 1.78	0.105	-12.17	-20.27 – -4.07	0.003			
Age ²	-0.26	-0.55 – 0.03	0.077				-1.20	-1.58 – -0.83	<0.001
Random Effects									
σ^2	230697.48			187089.40			264500.35		
τ_{00}	210658.79	Individual		230939.36	Individual		194982.18	Individual	
ICC	0.48			0.55			0.42		
N	825	Individual		419	Individual		406	Individual	
Observations	2111			979			1132		
Marginal R ² / Conditional R ²	0.059 / 0.508			0.005 / 0.555			0.022 / 0.437		

Note: MA = Middle age cohort; OA = Older age cohort; All = Both cohorts combined; HCV = Hippocampal volume; σ^2 = Residual random variance; τ_{00} = Individual intercept random variance; ICC = Intra-class correlation between individuals; Model S1 shows impact of age on total hippocampal volume for both cohorts; Model S2 shows impact of age on MA total hippocampal volume; Model S3 shows impact of age OA total hippocampal volume.

Figure S2 Total hippocampal volume trajectories for middle age, older age and all cohorts combined. The solid line is the combined trajectory (Table S2 Model S1), the dotted line is the middle age trajectory (Table S2 Model S2), and the dashed line is the older age trajectory (Table S2 Model S3). The individual data points are represented by dots.

Table S3 Goodness of fit characteristics for latent class sets by cohort and side.

Cohort	Side	N	AIC	BIC	loglik	npm	Class size					Posterior Probability					Posterior Probability > 0.7 (%)				
							C1	C2	C3	C4	C5	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
MA	LHC	1	14077.37	14093.52	-7034.68	4	419					100					100				
		2	14080.87	14109.13	-7033.43	7	398	21				0.9279	0.6543				96.48	33.33			
		3	14074.21	14114.59	-7027.11	10	287	100	32			0.8549	0.8449	0.882			84.67	79	90.62		
		5	14084.65	14149.26	-7026.33	16	155	145	83	32	4	0.5292	0.4936	0.7796	0.8698	0.3775	5.81	0	68.67	90.62	0
	RHC	1	14002.89	14019.04	-6997.45	4	419					100					100				
		2	14002.56	14030.83	-6994.28	7	216	203				0.7826	0.7446				70.83	59.11			
		3	14004.98	14045.36	-6992.49	10	250	150	19			0.7635	0.7896	0.7156			74	69.33	57.89		
		4	14010.98	14063.47	-6992.49	13	250	150	19	0		0.7635	0.7896	0.7156	0		74	69.33	57.89	0	
		5	14016.63	14081.24	-6992.32	16	250	149	19	1	0	0.7649	0.776	0.732	0.4962	0	73.2	67.11	57.89	0	0
OA	LHC	1	16312.81	16328.84	-8152.41	4	406					100					100				
		2	16303.72	16331.76	-8144.86	7	258	148				0.7517	0.7192				62.4	53.38			
		3	16304.98	16345.05	-8142.49	10	249	139	18			0.7459	0.6549	0.6093			61.85	38.85	33.33		
	RHC	1	16460.27	16476.3	-8226.14	4	406					100					100				
		2	16458.57	16486.61	-8222.28	7	278	128				0.7015	0.6705				50.36	34.38			
		3	16462.58	16502.65	-8221.29	10	297	65	44			0.6824	0.5838	0.6115			49.49	20	25		

Notes: classes that did not converge have been omitted from the table; MA = Middle age cohort; OA = Older age cohort; LHC = Left hippocampus; RHC = Right hippocampus; AIC = Akaike information criterion; BIC = Bayesian information criterion; loglik = log-likelihood; npm = number of parameters; Cn = Class number;

Table S4 Latent Class characteristics at Baseline

	Middle Age						Older Age				
	Left Hippocampus					Right Hippocampus			Left Hippocampus		
	Class 1	Class 2	C1 vs C2	Class 3	C1 vs C3	Class 1	Class 2	C1 vs C2	Class 1	Class 2	C1 vs C2
No.	283	98		32		213	200		243	139	
Age, years (SD)	47.19 (1.37)	47.07 (1.35)	p=0.473 ^a	47.89 (1.39)	p=0.005^a	47.31 (1.39)	47.11 (1.37)	p=0.148 ^a	63.11 (1.4)	63.08 (1.48)	p=0.819 ^a
Male, No. (%)	125 (44.17)	39 (39.8)	p=0.451 ^b	21 (65.62)	p=0.011^a	105 (49.3)	80 (40)	p=0.058 ^b	123 (50.62)	76 (54.68)	p=0.445 ^b
Years of Education, (SD)	14.76 (2.2)	15.06 (2.27)	p=0.257 ^a	14.84 (2.39)	p=0.653 ^a	14.84 (2.14)	14.83 (2.33)	p=0.957 ^a	14 (2.66)	14.41 (2.53)	p=0.151 ^a
BMI, kg/m ² (SD)	27.26 (4.89)	26.43 (4.63)	p=0.146 ^a	27.6 (4.76)	p=0.246 ^a	27.12 (4.78)	27.05 (4.89)	p=0.894 ^a	26.45 (4.44)	26.29 (4.23)	p=0.728 ^a
Hypertension, No. (%)	82 (28.98)	21 (21.43)	p=0.147 ^b	10 (31.25)	p=0.258 ^b	63 (29.58)	50 (25)	p=0.297 ^b	142 (58.44)	85 (61.15)	p=0.603 ^b
Diabetes, No. (%)	8 (2.83)	1 (1.02)	p=0.310 ^b	1 (3.12)	p=0.401 ^b	8 (3.76)	2 (1)	p=0.069 ^b	19 (7.82)	13 (9.35)	p=0.603 ^b
<i>APOE</i> -ε4 carrier, No. (%)	73 (25.8)	30 (30.61)	p=0.355 ^b	11 (34.38)	p=0.691 ^b	53 (24.88)	61 (30.5)	p=0.202 ^b	50 (20.58)	38 (27.34)	p=0.131 ^b
Smoker, No. (%)	40 (14.13)	19 (19.39)	p=0.215 ^b	4 (12.5)	p=0.375 ^b	30 (14.08)	33 (16.5)	p=0.495 ^b	17 (7)	9 (6.47)	p=0.846 ^b
MMSE, score (IQR)*									30 (1)	30 (1)	p=0.457 ^c

Notes: BMI = body mass index; MMSE = Mini-mental state examination; C1 vs C2 = Class 1 to Class 2 comparison; C1 vs C3 = Class 1 to Class 3 comparison;

* MA cohort was not assessed using the MMSE test; a = t-test; b = χ^2 test; c = Wilcoxon signed-rank test.

Table S5 Older Age Latent Class Characteristics at baseline, excluding MMSE < 27

	Left Hippocampus		
	Class 1	Class 2	C1 vs C2
No.	222	146	
Age, years (SD)	63.1 (1.43)	63.13 (1.46)	p=0.877 ^a
Male, No. (%)	111 (50)	78 (53.42)	p=0.520 ^b
Years of Education, (SD)	14.04 (2.61)	14.49 (2.51)	p=0.109 ^a
BMI, kg/m ² (SD)	26.46 (4.45)	26.25 (4.26)	p=0.647 ^a
Hypertension, No. (%)	129 (58.11)	88 (60.27)	p=0.679 ^b
Diabetes, No. (%)	17 (7.66)	14 (9.59)	p=0.514 ^b
<i>APOE</i> -ε4 carrier, No. (%)	45 (20.27)	41 (28.08)	p=0.083 ^b
Smoker, No. (%)	15 (6.76)	10 (6.85)	p=0.972 ^b
MMSE, score (IQR)	30 (1)	30 (1)	p=0.583 ^c

Notes: BMI = body mass index; MMSE = Mini-mental state examination;

C1 vs C2 = Class 1 to Class 2 comparison; a = t-test; b = χ^2 test; c = Wilcoxon signed-rank test.

Figure S3 Projected hippocampal volume from 20 to 80 years of age. The light dots are projected hippocampal volumes and the dark dots are observed hippocampal volumes. The dashed line is the projection for the right hippocampus and the solid line is the projection for the left hippocampus.

Table S6 Projected hippocampal volumes and atrophy from 20 to 80 years of age.

Age	<u>Total Hippocampus</u>			<u>Left Hippocampus</u>			<u>Right Hippocampus</u>		
	Vol (mm ³)	<u>Atrophy (%)</u>		Vol (mm ³)	<u>Atrophy (%)</u>		Vol (mm ³)	<u>Atrophy (%)</u>	
		Cumulative	Annual		Cumulative	Annual		Cumulative	Annual
20	6855			3410			3445		
30	6862	0.05%	0.01%	3411	0.11%	0.02%	3452	0.01%	0.01%
40	6818	0.70%	0.07%	3379	1.03%	0.09%	3438	0.40%	0.04%
50	6721	2.11%	0.14%	3316	2.88%	0.19%	3405	1.36%	0.10%
60	6572	4.28%	0.22%	3220	5.68%	0.28%	3351	2.91%	0.15%
70	6371	7.20%	0.29%	3093	9.41%	0.37%	3278	5.04%	0.21%
80	6118	10.89%	0.37%	2934	14.08%	0.47%	3185	7.74%	0.27%

* Total volume maximum is 6866 mm³ at age 26; Left volume maximum is 3414 mm³ at age 25; Right volume maximum is 3452 mm³ at age 29

Figure S4 Older age right hippocampal trajectories corresponding to the subgroups of the OA left 2 class latent class model (Class1 $n = 258$, Class2 $n = 148$) Class 1: Trajectory is represented by a solid line and the data points by circles, Class 2: Trajectory is presented by short dashed line and data points by triangles.

Table S7 Baseline characteristics of participants who remained in the study compared to those who dropped out.

	Older Age Left Hippocampus					
	Class 1			Class 2		
	Remain	Dropout		Remain	Dropout	
No.	127	116		83	56	
Age, years (SD)	62.84 (1.34)	63.41 (1.4)	p=0.001^a	63.04 (1.48)	63.13 (1.5)	p=0.715 ^a
Male, No. (%)	69 (54.33)	54 (46.55)	p=0.226 ^b	53 (63.86)	23 (41.07)	p=0.008^b
Years of Education, (SD)	14.25 (2.77)	13.75 (2.53)	p=0.155 ^b	14.69 (2.4)	14 (2.68)	p=0.128 ^b
BMI, kg/m ² (SD)	26.28 (4.5)	26.64 (4.39)	p=0.537 ^a	26.05 (4.04)	26.64 (4.51)	p=0.446 ^a
Hypertension, No. (%)	72 (56.69)	70 (60.34)	p=0.564 ^b	51 (61.45)	34 (60.71)	p=0.931 ^b
Diabetes, No. (%)	7 (5.51)	12 (10.34)	p=0.161 ^b	8 (9.64)	5 (8.93)	p=0.888 ^b
<i>APOE</i> -ε4 carrier, No. (%)	27 (21.26)	23 (19.83)	p=0.783 ^b	25 (30.12)	13 (23.21)	p=0.370 ^b
Smoker, No. (%)	6 (4.72)	11 (9.48)	p=0.146 ^b	5 (6.02)	4 (7.14)	p=0.793 ^b
MMSE, score (IQR)	30 (1)	30 (1)	p=0.126 ^c	30 (1)	30 (1)	p=0.640 ^c

Notes: BMI = body mass index; MMSE = Mini-mental state examination. a = t-test; b = χ^2 test; c = Wilcoxon signed-rank test.

References

- Shaw, M.E., Nettersheim, J., Sachdev, P.S., Anstey, K.J., Cherbuin, N., 2017. Higher Fasting Plasma Glucose is Associated with Increased Cortical Thinning Over 12 Years: The PATH Through Life Study. *Brain topography* 30(3), 408-416.
- Sled, J.G., Zijdenbos, A.P., Evans, A.C., 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE transactions on medical imaging* 17(1), 87-97.