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Subregional volumes of the hippocampus in relation to cognitive function and risk of dementia

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ABSTRACT

Background: Total hippocampal volume has been consistently linked to cognitive function and dementia. Yet, given its complex and parcellated internal structure, the role of subregions of the hippocampus in cognition and risk of dementia remains relatively underexplored. We studied subregions of the hippocampus in a large population-based cohort to further understand their role in cognitive impairment and dementia risk.

Methods: We studied 5035 dementia- and stroke-free persons from the Rotterdam Study, aged over 45 years. All participants underwent magnetic resonance imaging (1.5 T) between 2005 and 2015. Automatic segmentation of the hippocampus and 12 of its subregions was performed using the FreeSurfer software (version 6.0). A cognitive test battery was performed, and participants were followed up for the development of dementia until 2015. Associations of hippocampal subregion volumes with cognition and incident dementia were examined using linear and Cox regression models, respectively. All analyses were adjusted for age, sex, education, and total hippocampal volume.

Results: Mean age was 64.3 years (SD 10.6) with 56% women. Smaller volumes of the hippocampal fimbria, presubiculum and subiculum showed the strongest associations with poor performance on several cognitive domains, including executive function but not memory. During a mean follow-up of 5.5 years, 76 persons developed dementia. Smaller subiculum volume was associated with risk of dementia adjusted for total volume (hazard ratio per SD decrease in volume: 1.75, 95% confidence interval 1.35; 2.26).

Conclusions: In a community-dwelling non-demented population, we describe patterns of association between hippocampal subregions with cognition and risk of dementia. Specifically, the subiculum was associated with both poorer cognition and higher risk of dementia.

Introduction

Dementia is a complex and multifactorial syndrome, which includes disease entities such Alzheimer's disease, and is often characterized by gradual accumulation of brain pathology. This accumulation of pathology leads to disturbances in brain macrostructure, of which atrophy is the most apparent and for many forms is seen early on in the hippocampal formation. Indeed, hippocampal volume has been robustly associated to memory performance as well as risk of dementia, and is considered a reliable MRI-biomarker for progression of disease (Apostolova et al., 2006; den Heijer et al., 2010; Galton et al., 2001; Ikram et al., 2010).

Whilst most in vivo human studies linking hippocampus to dementia have focused on its gross volume, animal and pathological studies reveal the hippocampus to contain anatomical subregions with corresponding functional specialisation (Mueller et al., 2011; Tamnes et al., 2014; Yassa et al., 2011). It is conceivable that these subregions may also exhibit

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differential patterns in their association with cognitive performance in specific domains, as well as subsequent risk of dementia. This is supported by clinical and animal research displaying associations between the total hippocampus, memory, spatial navigation and executive function, and also hippocampal lesions and attention deficits (Burgess et al., 2002; Frodl et al., 2006; Nedelska et al., 2012; Serkova et al., 2016). Analysis of subregion volumes has been applied to memory neuroscience implicating subregions such as the CA1, CA3, and dentate gyrus to be important in memory (Mueller et al., 2011; Shing et al., 2011; Suthana et al., 2015; Tamnes et al., 2014; Yassa et al., 2010). However, to our knowledge the only subregion specific research outside memory links the dentate gyrus to spatial navigation (Kesner, 2013). Research additionally suggests differential trajectories for decline in different cognitive domains in dementia (Smits et al., 2015), with subjective cognitive impairment surfacing first (Burmester et al., 2016), then memory, followed by executive functioning and verbal fluency (Hamel et al., 2015). This hypothesis, and that of subregion functional specification, is supported by small studies suggesting a sequential pattern of atrophy starting within entorhinal and transentorhinal areas and moving to cornu ammonis area 1 (CA1), subiculum and eventually other subregions (Apostolova et al., 2010; Csernansky et al., 2005). However, such studies used shape or radial distance mapping to implicate specific subregions, whereas nowadays more robust and replicable segmentation algorithms are available (Iglesias et al., 2015). Another study demonstrates that neuronal loss in the CA1 is relatively limited in normal ageing (West et al., 1994), suggesting that the CA1 may provide information to differentiate healthy individuals from patients in an early disease state. Understanding how hippocampal subregion volumes link to cognition and risk of dementia can thus provide new pathophysiological insights and provide better understanding of the early trajectory of subregion atrophy in the development of dementia.

We therefore investigated the association of hippocampal subregions with cognitive function and risk of dementia within the population-based setting of the Rotterdam Study.

Methods and materials

Setting

The Rotterdam Study is a prospective population-based cohort that started in 1990 and included 14,926 participants, aged 45 years and older and living in Ommoord, a suburb of Rotterdam (Ikram et al., 2017). At study entry and at each follow-up visit (every 3–4 years), all study participants underwent extensive investigations at the dedicated research centre. From 2005 onwards, magnetic resonance imaging (MRI) of the brain was added to the core study protocol (the Rotterdam Scan Study) (Ikram et al., 2015). Since implementation of MR imaging within the study, 5689 persons successfully underwent MRI scanning from August 2005 until November 2013. We excluded persons with prevalent stroke or dementia (as defined below), or missing information on these (N = 415), leaving 5274 persons. Of these, 5035 persons also had data available on cognitive function and useable hippocampal segmentations, and 4768 had follow-up information for dementia.

The Rotterdam Study was approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

MRI acquisition and processing

Brain imaging was performed on a 1.5 T MRI scanner with an eight-channel head coil (GE Signa Excite, General Electric Healthcare, Milwaukee, USA), and included a T1-weighted (T1w), proton density-weighted (PDw), and fluid attenuation inversion recovery (FLAIR) sequence that were used for tissue segmentation. Details of the scan parameters have been described previously (Ikram et al., 2015). Specifically, the sequence of particular importance for the current study was the T1-weighted 3D fast RF spoiled gradient recalled acquisition in steady state with an inversion recovery pre-pulse (FASRSPGR-IR) sequence (Ikram et al., 2015). It consisted of TR = 700 ms, TE = 14 ms, matrix side of 192×256 and flip angle = 70 with a voxel size of $1 \times 1 \times 1$ mm. All participants were imaged on the same scanner with fixed protocol and imaging parameters.

Using an automated processing algorithm based on a k-nearest-neighbour-classifier for tissue segmentation on the T1w, PDw, and FLAIR sequences, images were segmented into grey matter, cerebrospinal fluid, normal appearing white matter and white matter lesions (de Boer et al., 2009; Vrooman et al., 2007). These tissue segmentations were visually inspected and corrected manually when needed (Ikram et al., 2015). Intracranial volume was defined as the sum of all brain tissue classes and cerebrospinal fluid.

Using the FreeSurfer software (version 6.0) (Iglesias et al., 2015), we automatically segmented the hippocampus on the T1w images into 12 subregions, amongst other neighbouring structures. Briefly, the algorithm is based on 15 *ex vivo* MRI scans of the hippocampal formation that were obtained at ultra-high resolution (average isotropic resolution of 0.13 mm). These images were manually labelled to distinguish between 12 different subregions of the hippocampus per hemisphere: the CA1, CA2/3, CA4, fimbria, dentate gyrus, hippocampal-amygdaloid transition area (HATA), tail, molecular layer, parasubiculum, presubiculum, subiculum and fissure (see Fig. 1). Outliers were checked within the dataset and excluded if outside the normal range. Robustness of measurements was assessed through the intraclass correlation coefficient (ICC) within a randomly selected subset of individuals that were re-scanned within 2 weeks (n = 85). ICCs ranged from 0.61 to 0.86 in hemispheric segmentation, averaged hemispheres resulted in higher ICC (0.81–0.88) (Supplementary Table 1). We did not include the fissure in our analyses due to its poor reliability within this set (right side ICC = 0.61) and past research (Whelan et al., 2016). The total volume (mL) for the hippocampal formation was also calculated. Further detailed information on these algorithms have been described elsewhere (Iglesias et al., 2015).

Assessment of cognitive function

A cognitive test battery of five tests was used in all participants to assess executive functioning, fine motor speed, information processing, and memory. These tests were performed at the baseline centre visits around the time of scan with a mean difference of 0.24 years. The letter-

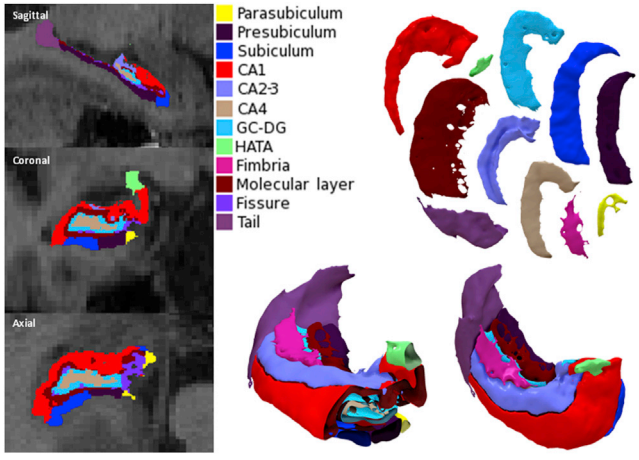

Fig. 1. Hippocampal sub region segmentation.
Figure displays the right Hippocampus on T1 MRI together with a 3D representation created via segmentation parameters. Abbreviations: CA, Cornu Ammonis, HATA, hippocampal-amygdaloid transition area.

digit substitution task (LDST) requires participants to make letter-digit combinations within 60 s (Bleecker et al., 1988). The Purdue pegboard test assesses bilateral fine manual dexterity using a pegboard with two rows of 25 holes to which the individuals have to place as many pins as possible into in a prescribed order in 30 s (Desrosiers et al., 1995). Three trials are carried out using right hand only, left hand only and both hands, with a summary score calculated. The abbreviated Stroop tests -reading, colour naming and interference-consists of naming printed words, naming printed colours, and naming the colour in which a colour-name is printed, respectively (Goethals et al., 2004; Golden, 1976). The word learning tests (15-WLT immediate recall, delayed recall and recognition trials) is based on Rey's auditory recall of words (Bleecker et al., 1988). Individuals are presented with 15 words and are asked to recall as many as possible (immediate), and also 15 min later (delayed). Recognition was tested via presenting these 15 words intermixed with a further 30 new words to which participants were asked if they recognised the words presented to them in the earlier trial. The word fluency test (WFT) tests verbal fluency through naming as many animals as possible within 60 s (Welsh et al., 1994). Stroop tests were inverted therefore better function is indicated by a higher score on all tests.

Using a principal component analysis we derived a measure of global cognition, the g-factor, as previously described (Hoogendam et al., 2014). This included a combination of standardized scores of LDST, Purdue pegboard test, Stroop interference, 15-WLT delayed, and WFT. The g-factor explained 49% of the total variance in cognition within the sample, which is expected (Hoogendam et al., 2014).

Assessment of incident dementia

Participants were screened for dementia using a protocol at baseline and at follow-up examinations. Initially, all individuals underwent the Mini-Mental State Examination and the Geriatric Mental Schedule organic level, with cut offs of below 26 and above 0 respectively (Copeland et al., 1976; Folstein, 2007). Screen-positives then underwent a physician interview and additional testing using the Cambridge examination for mental disorders in the elderly (Roth et al., 1986). In addition to this, the Rotterdam Study cohort is continuously monitored for numerous disorders and events, including dementia, through digital linkage with general practitioner records and the regional institute for outpatient mental health care. An adjudicated consensus panel (headed by a consultant neurologist) decided on the final diagnosis in all cases using the DSM-III-R criteria for all-cause dementia. NINCDS-ADRDA was used for the subtype of Alzheimer's Disease (American Psychiatric and American Psychiatric, 1994; McKhann et al., 1984; Schrijvers et al., 2012). When needed, clinical neuroimaging was used as an aid in determining the subtype of dementia and ruling out other causes. Follow-up for dementia was virtually complete until 2015.

Other measurements

Education was defined based on number of years spent in education. Information on cardiovascular risk factors and medication use was gathered through interviews and physical examinations (Ikram et al., 2017). Fasting serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and glucose levels were measured via an automated enzymatic procedure (Boehringer Mannheim system). Blood pressure (mmHg) was measured twice with a random-zero sphygmomanometer at the right arm. The average of the two measurements was used in the analyses. Smoking was categorized as current, former or never. Diabetes mellitus was defined as a fasting glucose level of $\geq$7 mmol/L, or if unavailable, non-fasting glucose level of 11.1 mmol/L and/or the use of anti-diabetic medication. Body mass index (BMI) was calculated as weight (kg)/height^2(m). *APOE* genotype was determined by polymerase chain reaction on coded DNA samples in the original cohort, and by bi-allelic Tacqman assays (rs7412 and rs429358) for the expansion cohorts.

Data analysis

The hippocampal subregions were segmented by hemisphere. Initial analyses did not reveal differences in left and right associations to cognitive function, prompting us to use the average in all further analyses.

The association of the hippocampal subregions with cognition and incident dementia was modelled using linear and Cox regression models, respectively. All analyses were adjusted for age, sex, education and intracranial volume (Model I), and the total volume of the hippocampal formation (Model II). In an additional step, the analyses were adjusted for cardiovascular risk factors including; HDL cholesterol, total cholesterol, lipid lowering medication, antihypertensive drugs, systolic blood pressure, diastolic blood pressure, smoking, diabetes mellitus, and BMI (Model III). Multicollinearity was checked using the variance inflation factor (Montgomery et al., 2012), and many subregions displayed high VIF values when adding total hippocampal volume to the models (Models II and III). Therefore, we first regressed out the effect of hippocampal volume on the subregions and subsequently used the standardized residuals in models II and III. This precluded any collinearity in these models, while also removing any interdependency between these variables. The robustness of our results was assessed by randomly splitting the study sample 60% and 40% and re-running model II. This was carried out 500 times and we reported the resulting averaged beta over the 500 samples and 95% range of these betas.

We also sought to explore time-specific patterns of association of the hippocampus and its subregions with incident dementia. We argued that those in an advanced stage along the trajectory of damage are closer to diagnosis and therefore would be expected to develop dementia already after a short follow-up. We therefore studied whether the effects of the subregions associated to risk of dementia varied according to the time to diagnosis. To this end, we used a sliding window approach in which we restricted the follow-up to a two-year time-window, which was then shifted sequentially in steps of six months to cover the complete follow-up time. This resulted in a total of twelve windows showing how hippocampal subregions linked to dementia, in 0.5 year's increment from 0–2 to 6–8 years after MRI. In other words, the first three windows were 0–2 years, 0.5–2.5 years, and 1–3 years after baseline. Hazard ratios therefore represent the risk of a subsample of those with a follow-up period within this time window. Risk factors used within this analysis are only included at baseline. The results were depicted in a figure showing hazard ratios from Model II with linear trend lines fitted to these results.

We repeated the analyses for Alzheimer's Disease (AD) only and stratifying by gender, age (above versus below 75, due to case numbers in younger participants), and *APOE* ε4 genotype. Tests were adjusted for multiple comparisons of the number of subfields using a Sidak correction. The number of independent tests were estimated which resulted in a significance threshold at $p < 0.004$ corrected value (Šidák, 1967).

Results

The mean age of the population was 64.3 years (SD 10.6) with 56% women (Table 1). Mean hemispheric hippocampal volume (average of both hemispheres) was 3.33 ml; the mean volume of the hemispheric CA1 (average of both hemispheres), the largest subregion, was 0.61 ml.

Hippocampal subregions and cognition

Total hippocampal volume was associated with all cognitive tests except word learning tests (Supplementary Table 3).

All subregions, except for parasubiculum, were associated with the g-factor in Model I (Supplementary Table 4). After adjustment for total hippocampal volume, no subregions remained significantly associated with the g-factor to multiple testing threshold (Fig. 2A, Supplementary Tables 5 and 6).

Table 1
Baseline characteristics of the study population.

Characteristic	N = 5035
Age, years	64.33 (10.58)
Women	55.6% (2836)
Education, years	12.64 (3.91)
Cardiovascular factors	
HDL cholesterol, mmol/l	1.52 (2.50)
Total cholesterol, mmol/l	5.58 (1.96)
Lipid lowering medication	24% (1215)
Antihypertensive medication	31.7% (1614)
Systolic blood pressure, mm Hg	141.42 (39.53)
Diastolic blood pressure, mm Hg	84.37 (37.37)
Smoking	
Former	48.9% (2495)
Current	19.8% (1010)
Diabetes mellitus	11.4% (580)
Body mass index, kg/m^2	27.48 (4.16)
APOE e4 carriership	
1	25% (1254)
2	2% (106)
Hippocampal volumes	
Total hippocampal volume, ml	3.33 (0.40)
Subregions	
CA1, ml	0.61 (0.08)
CA2/3, ml	0.21 (0.03)
CA4, ml	0.25 (0.03)
Fimbria, ml	0.07 (0.02)
Dentate gyrus, ml	0.29 (0.04)
HATA, ml	0.06 (0.01)
Tail, ml	0.52 (0.07)
Molecular layer, ml	0.55 (0.07)
Parasubiculum, ml	0.07 (0.01)
Presubiculum, ml	0.31 (0.04)
Subiculum, ml	0.41 (0.05)

Categorical variables are presented as percentages (numbers); continuous variables as means (standard deviations). Abbreviations: CA: Cornu Ammonis, HATA: hippocampal-amygdaloid transition area, HDL: high-density lipoprotein, ml: millilitres, *APOE*: Apolipoprotein E. Missing data on 7% for *APOE* e4 carriership.

Further investigation of the separate cognitive tests revealed more specific associations for the various subregions after adjusting for total volume (Supplementary Tables 4–6, Fig. 2B). Noteworthy results significant to multiple testing threshold include the presubiculum and its robust associations with a broad range of tasks. This was seen in LDST (Beta, 95% confidence interval) (−0.29, –0.46; –0.12), Purdue pegboard test (−0.21, –0.33; –0.09), and the Stroop task naming (−0.19, −0.32; –0.06). The subiculum was also associated the Stroop interference (−0.88, –1.47; –0.31) with strong trends displayed in numerous other tests. These associations were found to be robust through the split sample analysis (Supplementary Table 7). Associations attenuated after additionally adjusting for cardiovascular risk factors with full results in Supplementary Tables 4–6.

Hippocampal subregions volume and incident dementia

During a mean follow-up of 5.5 years, 76 persons developed dementia of which 49 were diagnosed with AD. Smaller volume of the total hippocampal formation was associated with higher risk of dementia (HR per SD decrease of whole volume 2.36, CI 95% 1.81; 3.07). In model I, all subregions except for the parasubiculum surpassed the threshold for statistical significance after controlling for multiple comparisons (Table 2). After adjusting for the total hippocampal volume via use of standardized residuals in model II we noticed that the effect for subiculum attenuated (HR 1.67, CI95% 1.30; 2.14) but remains significant whilst for other subregions the effect attenuated below the threshold for statistical significance. Goodness of fit was tested via the Likelihood Ratio Test displaying good indices of fit. Model III saw further mild attenuation however this model performed significantly better than II ($p < 0.05$) with a better measure of goodness of fit.

Sliding window analysis

In the sliding time-window approach (Fig. 3), we found that the risk of dementia related to total hippocampus volume showed the strongest effect in the first window (mean follow-up time 1.97 years: HR per SD 3.14 (2.02; 4.85)) and steadily decreased thereafter (mean follow-up time 6.89 years: HR per SD 1.49 (0.54; 4.09)) (Fig. 3). For the subiculum a similar pattern was seen. However, in contrast when adjusting the subiculum for the total hippocampus volume the effect remained

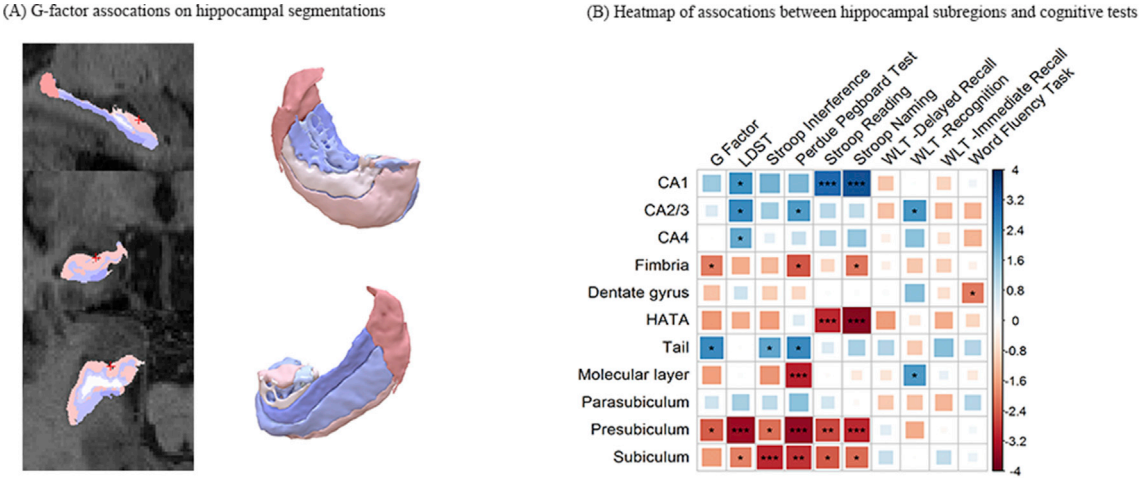

Fig. 2. Association between hippocampal subregions and cognitive tests.
(A) Association with g-factor per subregions are shown in the 3-dimensional position in the hippocampus with colour indicating direction of effect for larger volume of that subregion (blue: positive and red: negative) and statistical significance (more intense reflects smaller p-value) of the association. (B) Heatmap of associations between hippocampal subregion volume decrease and g-factor and separate cognitive tests. Colours and sizes of the blocks correspond to t-values, with blue and red indicating positive and negative associations, respectively. Larger blocks indicate stronger associations, and significance levels as indicated by asterisks: *$p < 0.05$ **$p < 0.01$ ***$p < 0.004$.
Both panels display results from Model II, adjusted for age, sex, education years, intracranial volume, and total hippocampus volume through use of standardized subregion residual term. Abbreviations: CA, Cornu Ammonis, HATA, hippocampal-amygdaloid transition area. Higher scores indicate better cognitive performance.

Table 2
Association between hippocampal subregion volume and the risk of dementia.

	Hazard ratios (95% confidence intervals) of the association of total hippocampus volume and its subregions with risk of dementia		
	Model I	Model II	Model III
Total hippocampus	*2.36 (1.81; 3.07)*	NA	*2.54 (1.93; 3.36)*
Subregions			
CA1	*2.09 (1.61; 2.73)*	0.92 (0.74; 1.14)	0.95 (0.76; 1.19)
CA2/3	*1.59 (1.21; 2.09)*	0.72 (0.56; 0.92)	0.71 (0.54; 0.93)
CA4	*1.92 (1.48; 2.50)*	0.78 (0.62; 0.99)	0.76 (0.60; 0.98)
Fimbria	*1.60 (1.24; 2.06)*	1.09 (0.88; 1.37)	1.13 (0.89; 1.44)
Dentate gyrus	*2.03 (1.55; 2.066)*	0.79 (0.63; 0.99)	0.78 (0.62; 0.99)
HATA	*1.78 (1.36; 2.33)*	1.00 (0.81; 1.24)	1.04 (0.82; 1.33)
Tail	*1.77 (1.34; 2.34)*	0.86 (0.68; 1.09)	0.82 (0.64; 1.05)
Molecular layer	*2.49 (1.90; 3.27)*	1.15 (0.90; 1.47)	1.24 (0.96; 1.59)
Parasubiculum	1.33 (1.06; 1.66)	1.05 (0.85; 1.29)	1.01 (0.80; 1.26)
Presubiculum	*2.52 (1.91; 3.32)*	1.33 (1.04; 1.69)	1.30 (1.01; 1.68)
Subiculum	*2.94 (2.22; 3.88)*	*1.67 (1.30; 2.14)*	*1.75 (1.35; 2.26)*

Hazard ratios represent increase in risk per standard deviation decrease in volume.
Model I: adjusted for age, sex, education and intracranial volume.
Model II: additionally adjusted for total hippocampal volume through use of the residual term.
Model III: additionally adjusted for diabetes, antihypertensive drugs, systolic blood pressure, diastolic blood pressure, lipid lowering medication, high-density lipoprotein cholesterol, total cholesterol, BMI, smoking.
Italic represents significance at Sidak multiple correction value of $p = 0.004$.
Abbreviations: CI: confidence interval. CA, Cornu Ammonis, HATA, hippocampal-amygdaloid transition area.

stable over the follow-up period.

Stratified analysis

Associations to only Alzheimer's dementia disease risk were similar to the all cause dementia. Hazard ratio per SD decrease of volume (CI 95%) was 2.55 (1.81; 3.57) for the whole hippocampal volume and 1.84 (1.35; 2.51) for subiculum (Supplementary Table 8). Further sensitivity analyses stratifying by sex, age (above versus below 75 years), and *APOE* ε4 genotype displayed no indication for effect modification by these factors (data not shown).

Discussion

In a large sample of community-dwelling adults, we found that volumes of hippocampal subregions had distinct associations to a range of cognitive tasks. Regions such as the presubiculum and subiculum were associated to a broad range of tasks themselves, such as Perdue pegboard task and Stroop subtasks. Moreover, we found that smaller volumes of the subiculum subregion were strongly associated with risk of incident dementia.

Potential limitations of the study should be mentioned. Firstly, our sample source consisting of a middle-class population from mainly white descent may restrict generalisation. Additionally, although the scans used were acquired to current clinical standards (3D acquisition, sub-mm voxels), and reproducibility for the segmentations was reasonably high, 1.5 T imaging is not ideally suited for parcellations of the subfields of the hippocampus. Higher resolution images with smaller voxel size or at higher field strength (e.g. 7 T) are available nowadays and would allow for a more accurate segmentation of the subregions. Thus, further studies with higher resolution should seek to replicate these findings. However, despite this use of T1w imaging for hippocampal segmentations with FreeSurfer 6.0 has previously been found to have a high level of test-retest reliability with 20 of the regions having an ICC greater than 0.9, including the CA1 and subiculum (Worker et al., 2018).

Strengths of the study include using a general population sample within a prospective design rather than a clinical cohort or highly selected individuals, as is seen in past research (Mueller et al., 2007). This reduces the likelihood of selection effects and allows for extensive adjustment for confounding. Additionally, in comparison to past research we extended the scope of the analysis, taking a wider range of subregions of the hippocampus into consideration. This was done through the use of the latest FreeSurfer method with improved segmentation (Iglesias et al., 2015).

The strongest and most robust association found for dementia risk, over and above that of total volume, in our study was with the subiculum. Interestingly, the effect of subiculum on dementia remained stable over the entire duration of follow-up. Previous findings, in small longitudinal and clinical cross-sectional samples, also found the subiculum to be a prominent subfield indicating risk for later development of dementia, together with the CA1 (Apostolova et al., 2010; Carlesimo et al., 2015). Other research specifically found the CA1 to be relatively spared in normal aging whilst affected in dementia, thus suggesting it to be an important marker in distinguishing between normal aging and early stages of dementia pathology (West et al., 1994). However, we found the CA1 was not related to risk of dementia whatsoever when adjusting for total hippocampal volume. This could suggest the subiculum as an earlier and more reliable marker for prediction of dementia, with the CA1 important in detecting and monitoring neurodegeneration later in the trajectory. This is plausible as the CA1 is known to be affected by amyloid and tau pathology. Therefore, the subiculum could provide information about risk before this pathology appears, yielding an earlier marker for dementia risk as was also evidenced by our sliding window analysis. Still, it is also possible that the discordance in results across various subregions is due to segmentation differences applied in earlier software algorithms,

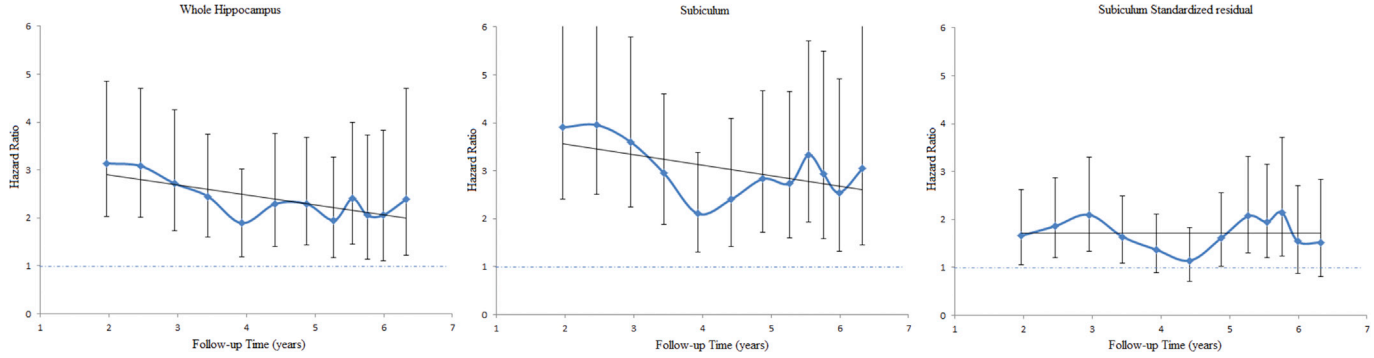

Fig. 3. The association of hippocampus and hippocampal subregion the subiculum with risk of dementia, by follow-up time.
Values represent hazard ratios, with error bars at 95% confidence interval, calculated for two-year intervals that slide towards longer follow-up time in 6-month increments. Values are plotted at the mean follow-up time for each window and represent change per standard deviation decrease in volume. Solid lines are linear trend lines fitted over the individual effect estimates. All values were adjusted for age, sex, education and intracranial volume.

which might have affected the segmentation boundary between CA1 and subiculum. Despite, recent methods being hailed to be more accurate based on a higher level of agreement with histological studies, there is still the possibility of inaccurate segmentation which could explain the lack of relationship to CA1 volumes (Iglesias et al., 2015). Additionally, whilst displaying robust ICC values the CA1 did display lower accuracy than the subiculum which could partly explain the results (Supplementary Table 1).

Another important finding is that hippocampal subregions were associated to a range of cognitive functions. This is particularly interesting with respect to dementia, in which the subclinical state is believed to involve focal hippocampal atrophy and deficits reasonably restricted to memory function. However, the current findings do not support this restriction to memory deficits, suggesting a broad range of cognitive changes may accompany the hippocampal alterations in these earlier stages. For example, whilst also strongly associated to dementia risk, subiculum and also presubiculum, were seen to be related to a wide range of cognitive tasks. This may be, in part, due to their anatomical connections and location within functional pathways. For instance, it is suggested that the medial entorhinal cortex projection, to which the presubiculum is thought to project to, receives most of the visual input (Caballero-Bleda and Witter, 1994; Ferbinteanu et al., 1999; Witter et al., 1989). Thus, adequate processing and projection of visual information may be an underlying process associating this region to a range of tasks. Similarly, the subiculum is the origin of not only many hippocampal projections to extrinsic sites but also other hippocampal subregions, directly and indirectly (Lavenex and Amaral, 2000). More specific tasks are needed to fully understand these relationships.

Past literature suggests associations between the CA1, CA3, CA4 and dentate gyrus with memory (Pereira et al., 2013; Suthana et al., 2015; Yassa and Stark, 2011). However, in the current research only trends in associations between subregions and memory were observed once controlling for total volume. The memory tasks used in our study have been cited to test multiple processes involved in memory such as consolidation and retrieval, but also attention which has also been linked to the hippocampus (Aly and Turk-Browne, 2017; Schoenberg et al., 2006). It is therefore possible that after adjusting for the total volume of the hippocampus, the association with our memory-test attenuates due to the other processes needed to complete the task being affected by different regions. Indeed, in models unadjusted for total hippocampus volume memory subtasks, especially delayed recall, were related to the aforementioned subregions. Conversely, this also means that the memory test used in our study was not specific enough to capture processes that have been previously linked to these subregions such as pattern completion and separation (Bakker et al., 2008; Yassa et al., 2011; Yassa and Stark, 2011). As noted tasks investigating more subtle underlying processes may be needed to disentangle this. A further suggestion is that the differences in segmentation reliability and accuracy between sub regions could be affecting the results (Mueller et al., 2011; Suthana et al., 2015; Yassa et al., 2010).

One possible link between the range of cognitive functions related to the subiculum, and the early changes in volume associated to later risk of dementia, is that of subjective cognitive impairment. Subjective cognitive impairment is thought to be an initial stage of later dementia where individuals complain about a range of cognitive function loss (Burmester et al., 2016; Reisberg et al., 2008). Subjective cognitive complaints have been suggested to be influenced by a range of other factors, for example psychosocial factors and reporting bias thus interpretation must be done with caution. Despite this, subjective cognitive impairment has been related to lower volume of the left hippocampus and subregions, CA1 and subiculum (Cantero et al., 2016; Perrotin et al., 2015; Saykin et al., 2006; van der Flier et al., 2004). Thus, more research regarding hippocampal subregion volume, subjective complaints and conversion into mild cognitive impairment and dementia is needed.

To conclude, our results implicate the subiculum as an important marker for dementia and also for further understanding the progression and trajectory of neurodegeneration beyond the total hippocampal volume. Furthermore, we found a wide range of associations between the hippocampal subregions and cognitive domains. Interestingly, CA1 was found to be non-predictive in cognition and dementia after adjusting for total volume; however this could possibly be due to inaccurate segmentation or low resolution. Despite this, the results suggest that the role of the hippocampal formation, and subregions involved in dementia, extend beyond memory. Taken together, our results propose that a more fine-grained analysis of brain structure could aid in the understanding of cognitive function and dementia.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2018.05.041.

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