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Structural and resting-state MRI detects regional brain differences in young and mid-age healthy APOE-e4 carriers compared to non-APOE-e4 carriers

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Abstract—The presence of the e4 allele of apolipoprotein E (APOE) gene is the best known genetic risk factor for Alzheimer’s disease. In this study we investigated the link between functional and behavioural differences and regional brain volume and cortical thickness differences between those that carry the e4 allele (e4+) and those that only carry the e3 allele (e3/e3). We studied these genotype populations in two age groups: a young group (average age 21 years); and a mid-age group (average age 50 years). High-resolution T1-weighted MRI scans were analyzed with Freesurfer to measure regional white matter brain volume and cortical thickness differences between genotype groups at each age. This data was correlated against behavioural findings on the same cohort. Resting-state MRI was also conducted to identify differences in underlying brain functional connectivity. We found that there is a positive correlation between the thickness of the parahippocampal cortex in young e4+ and performance on an episodic memory task. Young e4+ also showed a positive correlation between white matter volume in the left anterior cingulate and performance on a covert attention task. At mid age, e4+ had structural differences compared to e3/e3 in these areas: the parahippocampal cortex was thicker and white matter volume in the left anterior cingulate was greater than e3/e3. We discuss the possibility that an over-engagement with these regions by e4+ in youth may have a neurogenic effect that is observable later in life. The cuneus appears to be an important region for APOE-driven differences in the brain, with greater functional connectivity among young e3/e3 and greater white matter volume in the young e4+.

Index Terms—apolipoprotein E e4; cortical thickness; white matter volume; resting-state MRI; regional brain volume; neurogenesis.

I. INTRODUCTION

PERHAPS the greatest challenge facing health services around the world is the rise in age-related memory loss and dementia. The global societal costs of dementia were estimated to be 818 billion USD in 2015 (1), and this is expected to rise to over 1 trillion USD by 2030. Given the enormity of this public health challenge, there is particular interest in understanding factors that influence Alzheimer’s disease (AD) risk and identifying early markers in the genesis of dementias. The best established genetic risk factor for AD is associated with the apolipoprotein E (APOE) gene (2-4), which codes for a protein that is responsible for lipid and cholesterol transport to cell membranes throughout the body.

The APOE gene exists in three different isoforms (alleles), labelled epsilon 2, epsilon 3 and epsilon 4 (henceforth e2, e3 and e4 respectively). Each person carries two alleles, the most prevalent is the e3 form and is present in 77% of the population, 25% carry the e4 variant, while 15% carry the e2 form (5). Carriers of a single e4 allele face a 2-3 fold increase in the risk of developing AD compared to those with a double-e3 configuration, while those with two e4 alleles have a dramatically increased risk of up to 15 times (6). In contrast, those carrying at least one e2 allele could see their risk of AD reduced by half (7). In this paper, those who carry at least one e4 allele are termed e4+, those who do not are e4-, while homozygous e3 carriers are termed e3/e3. The e4 allele has also been shown to affect cognitive performance in healthy ageing: e4+ show poorer performance on tests of memory function relative to e4- in later life (8,9). Longitudinal studies have shown that e4+ experience greater cognitive decline across the lifespan (10,11). The e4 allele has clear negative consequences in later life but, paradoxically, there is evidence that it might confer some cognitive advantages in young adulthood (12-14). This has led to the suggestion that the e4 allele might represent an example of antagonistic pleiotropy (15,16), where a gene impacts fitness differently across the lifespan. Under this hypothesis, mid adulthood would represent a transition point for e4+ as cognitive advantages give way to disadvantages. Although mid age likely represents the best time point to deliver intervention strategies to protect e4+ against imminent cognitive decline (17), most of the work exploring APOE effects on neural structure and function has focused on later life. In this work, we set out to explore APOE effects on brain structure in both young adulthood and mid-age. We also explored whether structural indices could be linked to cognitive performance, and analyzed APOE effects on measures of resting state functional brain activation.

As well as a focus on later life cognition, many neuroimaging studies of APOE effects have also paid particular attention to hippocampal structure and function, since AD pathology first becomes apparent in the hippocampal formation (18). In healthy older adults, some studies have shown e4+ to have smaller hippocampal volumes (19,20) although subsequent work found the effect to only be present longitudinally (20), restricted to homozygous e4+ (21), or not present at all (22,23). A small number of studies have examined similar

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effects in healthy young adults: one study has reported reduced hippocampal volume in e4+ aged 20-38 (24), another reported reduced entorhinal cortical thickness in adolescents (25), yet another study in adolescents found no effects (26). Thus, the link between APOE status and hippocampal volume in healthy ageing is by no means clear. Measures of cortical thickness in the hippocampal subregions have shown that e4+ aged 45-55 years suffer a thinning of the entorhinal cortex and subiculum: overall volume only differed at the trend level, suggesting that APOE effects could be more localised, which could help explain inconsistencies in findings (27).

Although there are many imaging studies that have investigated the structural links between grey matter and APOE status, there are comparatively few that have studied the white matter. However, in the last few years there has been rapidly-growing interest in this area and genotype-dependent white matter differences have been found that accompany grey matter. Recent studies (28-30) have shown that there is detectable white matter disruption in these areas in individuals in the prodromal stages of AD as well as neuronal degeneration and volume loss (31).

The parahippocampus is adjacent to the hippocampus and comprises the entorhinal and perirhinal cortices and, coupled with the posterior cingulate, plays an important role in memory processing and storage. Cortical thinning has been identified in the parahippocampal gyrus in AD (32) and in carriers of an APOE promoter gene a recent study suggests that this region is affected by genotype polymorphism (33). In addition, a recent resting-state study has suggested that the parahippocampal gyrus may exhibit deviant functional connectivity in cognitively normal elderly e4+ individuals (34). Studies exploring functional activation levels in the resting state have implicated the hippocampus as a locus for APOE effects (35-37); other studies have shown aberrant hippocampal activity in e4+ during memory tasks (35,38), and also during tasks that do not typically engage the hippocampus (14,39). Some authors have suggested that these effects relate to future AD risk, possibly reflecting early functional compensation. Resting state MRI studies have also pointed to differences in default mode network (DMN) activity. Altered coactivity within the DMN has been reported in both young adult (35) and healthy older e4+ (40), and is of significance because AD pathology seems to favour regions employed by the DMN (41), with AD patients showing disrupted DMN coactivity (42). Activity within the anterior cingulate cortex (ACC), a core node within the DMN, has been shown to be affected by APOE status. Using arterial spin labelling, it has been shown that cerebral blood flow (CBF) in the ACC is reduced in older e4+ but increased in young adult e4+. Furthermore, increased CBF in the left ACC was positively correlated with executive functioning in young adult e4+ only (43). Functional imaging studies have also pointed to the ACC as a potential locus for APOE related differences, perhaps driven by alterations in CBF. In individuals aged 49-75, it has been shown that e4+ under-recruit their ACC during a semantic categorisation task; abnormal responses in right hippocampus were also observed (44). In young to middle-aged adult e4+, reduced grey matter volume has been reported in ACC (45). The

posterior cingulate cortex also forms a core DMN node, and APOE effects have been reported in this region also, with e4+ (aged 49-67) showing reduced glucose metabolism (46). Enhanced connectivity between hippocampal and posterior cingulate regions has been reported in young adult e4+ (47).

Our recent work has suggested that another region, the cuneus, might also be a site whose activity might differentiate e4+ from e4- (48). Using functional MRI, we explored activation patterns during tasks of prospective memory and covert attention, in two age groups of healthy individuals. We tested e4+ and e3/e3 in young adulthood (aged 18-28) and mid age (aged 45-55). Results suggested a pattern of decreased activity in the cuneus in mid-age e4+ only, which we interpreted as reflecting accelerated neural ageing in e4+. Healthy elderly individuals show decreased activity in the cuneus (reliably demonstrated across a range of cognitive paradigms), thought to reflect impoverished processing of visual sensory input (49,50). Thus mid-age e4+ seem to show a pattern of neural recruitment normally seen in older adults, suggesting accelerated ageing. Interestingly, the cuneus seems to be one of the first sites where amyloid is deposited (51,52). PET studies have also shown that AD causes a shift in glucose metabolism in the brain, away from the cuneus to more frontal regions (53), again suggesting that the cuneus is affected early in AD disease progression. In the neighbouring precuneus, we found that healthy young adult e4+ showed a failure to deactivate during functional imaging of the covert attention task (14). The precuneus is also a key region in the DMN, and resting state studies have found disrupted precuneus connectivity in healthy older e4+ (54).

In spite of these differences between e4+ and e4-, there have been relatively few human studies that have correlated the behavioural and structural findings (55,56). A general link between brain anatomy and function might be expected since the efficiency of neural processing depends on the number, size and configuration of neurons, together with the number and type of synaptic connections they make (57). A recent study has uncovered the intriguing possibility that neuronal activity is a key factor in driving the growth of new neurons, and this effect has been identified in the hippocampal dentate gyrus and subventricular zone (58). This implies that differences in brain activation levels, such as those observed between e4+ and e4-, could stimulate changes in brain volume and structure.

In this work, we investigate whether structural MR imaging data can detect subtle regional brain volume or cortical thickness differences between e4+ and e3/e3, at two narrowly defined age ranges reflecting young adulthood and mid-age. Based on the findings outlined above, our a priori regions of interest were the key brain areas of parahippocampus, anterior cingulate, posterior cingulate, cuneus and precuneus. This study is not limited to grey matter regions only; in addition to measuring cortical thickness we also calculate white matter volume ratios in these areas, since there is a growing body of research that has identified subtle changes to the connecting white matter regions (29,30,59). Furthermore, a study of white matter has additional importance when considering the potential link between neurogenic processes and genetics-driven brain function. Given the important strong association

of hippocampal changes with aging and dementia, we study this brain area by additionally measuring the hippocampal grey matter volume ratio. We use behavioural data collected from the same individuals to investigate links between brain structure and behavioural performance, and assess the possible influence of APOE status. These behavioural data have been published elsewhere (14,48). We evaluated correlations between parahippocampal cortical thickness and performance on a 20-item word recall task. For the cuneus, precuneus and anterior cingulate, we used a measure of attentional switching performance derived from a covert attention task to investigate links between brain volume and behaviour in these regions. These measures were selected as being most appropriate given the putative functions of the regions under study. No correlations were assessed for the posterior cingulate, since there is insufficient evidence to link it to either task. Finally, we report data from resting-state MRI analyses in the same individuals. We hypothesized that the resting state analyses would show disrupted DMN coactivation as suggested by previous studies (60). We also hypothesized that resting state differences would co-localise with structural effects, which, alongside behavioural correlates, could point to the functional significance of any structural differences.

II. METHODS

A. Participants and genotyping

Ninety-eight healthy young participants were recruited (age 20 ± 2 years (mean $\pm$ SD), range 18 – 30 years, 64 females, 34 males), and 78 healthy mid-age participants were recruited (age 51 ± 3 years, range 43 – 58, 44 females, 34 males); all participants were right-handed. Volunteers were excluded from the study for untreated high blood pressure, cardiac pathology, a history of psychiatric or neurological illness, current use of psychoactive medication, and presence of metallic implants including bridges and braces, or tattoos above the shoulder. All participants were non-smokers (and had been for at least 5 years), had a body mass index within the normal range and had English as their first language. Initial screening involved signing of written informed consent (following procedures approved by the University of Sussex Schools of Psychology and Life Sciences Research Ethics Committee), and cheek swab samples were collected for DNA analysis from each participant. APOE genotypes were determined by KBiosciences (Hoddesdon, UK; www.kbioscience.co.uk), using their own system of fluorescence-based competitive allele-specific polymerase chain reaction (KASPar). Two APOE single-nucleotide polymorphisms (SNPs) rs429358 and rs7412 allowed identification of the three major APOE alleles (e2, e3 and e4).

1) *Young group*: From the samples, 9 participants were heterozygous e2 and excluded from the study. 50 were e3/e3, of which 20 were willing and eligible to participate; providing a control group representing the genotype most frequent in the general population (61). From the remaining 34 volunteers who were e4+, we were able to recruit 21 of the group to be participants to enter our experimental group. This group included two participants that were homozygous e4 carriers.

2) *Mid-age group*: From the anonymized samples, 9 participants were found to be heterozygous e2 and excluded from the study. Forty-seven were e3/e3, and the remaining 22 volunteers were e4+. All participants who coded as e4+ were invited to take part in the study as the experimental group. The same number of participants who coded for e3/e3 were recruited as the control group. Twenty individuals from the e3/e3 group, and 17 individuals from the e4+ group returned; this group included three homozygous e4 carriers.

Volunteers signed written informed consent, following procedures approved by the relevant Ethics Committees. The genotype results, imaging and behavioural data were anonymized and compiled by a third party so that neither volunteers nor the researchers were aware of the genotype results.

Demographics are shown in Table 1. Independent two-tailed t-tests demonstrated that there were no significant ($p > 0.05$) differences in age, IQ or years in education between our genotype groups at young age (Table 1a) or at mid age (Table 1b).

Table I
VOLUNTEER CHARACTERISTICS AND MEASURES OF BASELINE COGNITIVE PERFORMANCE FOR THE YOUNG AND MID-AGE GROUPS. STANDARD DEVIATIONS IN PARENTHESES.

Genotype Group	Age (years)	Gender	IQ	Years in education
<i>Young group</i>				
e4+ (n=21)	21.4 (2.2)	13F	113 (4)	15.1 (0.2)
e3/e3 (n=20)	20.9 (1.4)	14F	115 (3)	15.1 (0.3)
t statistic	t = 0.82		t = 0.02	t = 0.18
p value	p = 0.42		p = 0.23	p = 0.56
<i>Mid-age group</i>				
e4+ (n=17)	49.4 (3.9)	12F	119 (5)	15.0 (1.55)
e3/e3 (n=20)	50.5 (4.5)	11F	121 (4)	14.6 (1.57)
t statistic	t = 0.85		t = -1.97	t = 0.75
p value	p = 0.39		p = 0.10	p = 0.51

B. Magnetic resonance imaging protocol

All images were acquired on a Siemens 1.5 T Avanto MRI scanner (Siemens, Erlangen, Germany). High-resolution anatomical images were acquired using a 3D T1-weighted MPRAGE sequence, using TR = 1160 ms, TE = 4.44 ms, TI = 600 ms with a FOV = 230 x 230 mm². The acquisition matrix was 256 x 256, the flip angle = 15°, resulting in voxel dimension of 0.9 x 0.9 x 0.9 mm³. Acquisition time was 5 min. B0 gradient echo fieldmaps were acquired with TR = 513 ms, TE = 5.78 ms, FOV = 192 mm, acquisition matrix = 64 x 64 and a flip angle of 60°. The resulting voxel dimensions were 3 x 3 x 3 mm³. Acquisition time was 1 min. Resting state fMRI was acquired over a 7 minute period; participants were instructed to keep their eyes open and not fall asleep: no further instructions were given. There was no visual stimulation apart from the dim lighting in the scanner room. Thirty-six 3 mm slices (0.75 mm interslice gap) were acquired with an in-plane resolution of 3 mm x 3 mm (TR = 3300 ms per volume, TE = 50 ms).

C. Resting state fMRI analysis

Image pre-processing and analysis was conducted using FSL MELODIC and the FSL pre-processing tools. Pre-processing consisted of motion correction, brain extraction, spatial smoothing using a Gaussian kernel of full-width at half maximum (FWHM) of 5 mm, and a high-pass temporal filter was set to 150 s (0.007 Hz). Each participant's dataset of 130 volumes was then registered to their structural scan. Independent Component Analysis (ICA) as implemented in MELODIC was then used to decompose the temporally concatenated datasets into 20 independent components. The set of spatial maps from the group-average analysis was used to generate subject-specific versions of the spatial maps, and associated time series, using dual regression (35). First, for each subject, the group-average set of spatial maps is regressed (as spatial regressors in a multiple regression) into the subject's 4D space-time dataset. This results in a set of subject-specific time series, one per group-level spatial map. Next, those time series are regressed (as temporal regressors, again in a multiple regression) into the same 4D dataset, resulting in a set of subject-specific spatial maps, one per group-level spatial map. We then tested for main effects of genotype using FSL's randomise permutation-testing tool. Classification of resting state networks was performed by two independent raters. Results are presented at a significance threshold of $p < 0.05$ FDR-corrected.

D. Structural MRI analysis

In this study, we report measures of cortical thickness and white matter volume in the important brain regions of the cuneus (left+right hemisphere), anterior cingulate, and the parahippocampus (left+right hemisphere) shown in Figure 1. These metrics were obtained following cortical reconstruction and volumetric segmentation of subjects' T1-weighted MPRAGE images using the Freesurfer image analysis suite (<http://surfer.nmr.mgh.harvard.edu>). The reconstruction pipeline employed by Freesurfer includes motion correction (62) and the exclusion of non-brain tissue was performed using a hybrid watershed/surface deformation procedure (63). Images are transformed to Talairach space and the subcortical white matter and deep grey matter structures are segmented (64,65). Tessellation and surface deformation of the white/grey and grey/CSF border is employed to improve the reliability of the segmentation (66) and images are registered to a spherical atlas that matches individual cortical folding patterns to cortical geometry across subjects. This allows the extraction of cortical thickness (defined as the distance between grey/white matter boundary and the grey matter/CSF boundary) (66). To account for the differences in head size, we normalized the volume of segmented regions by dividing by the estimated total intracranial volume to give a volume ratio. Cortical thickness measures are not normalized since thickness measures do not scale linearly with head size. In only 4 subjects, minor manual edits were required to improve the accuracy of the pial and white matter surfaces in the temporal lobes as estimated by Freesurfer. Cortical thickness and white matter volumes for 6 regions (parahippocampus,

cuneus and left and right anterior cingulate, precuneus and posterior cingulate) were compared statistically using a two-tailed t-test (equal variances assumed) in SPSS version 22. An adjusted (Bonferroni-corrected) significance level of $p = 0.008$ was used for each region. Age and gender were included as covariates of no interest in the statistical analysis to account for any gender by genotype interactions. However, such effects are mainly observed in older individuals (60+ years) (17) and we did not anticipate any effects on either of our cohorts.

E. Correlation with behavioural measures

Localized volume and thickness changes in the cuneus, precuneus, parahippocampus, and anterior cingulate were correlated with behavioural indices that are known to recruit these specific brain areas. The posterior cingulate is not strongly associated with either the memory or covert attention tasks performed in this study, and was not included in this analysis. The behavioural data used for the correlations are summarized in Table 2. This data has been published elsewhere (14,48) together with a detailed statistical analysis and discussion of the genotype- and age-dependent differences that were observed. In summary, a main effect for age was identified in the covert attention task ($p = 0.001$) together with a main effect of genotype in the mid-age group ($p = 0.039$) with e4+ showing better task performance.

Given the well-established role of the hippocampus in supporting memory, we correlated episodic memory performance and parahippocampal thickness. Episodic memory was measured using immediate free recall of a supraspan unrelated word list. Participants are presented with 20 unrelated words on-screen at a rate of one word every two seconds; after all 20 words have been presented they are prompted for immediate written recall.

The cuneus and anterior cingulate has been shown to be recruited during a covert attention (CA) task (67). Thus, we correlated attentional switching and anterior cingulate and cuneus volume. The CA task (based on (67)) is a computer-based task which involves target detection following a cue. In each trial, participants fixate a small diamond in the centre of the screen; after a 3000ms delay one side of the diamond changes colour briefly, cueing either left or right. The target is then displayed to either the left or right of the diamond. The cue is predictive in 70% of trials (cue congruent), and incorrect for the remaining 30% of trials (cue incongruent). Participants indicate with a left or right button response which side of the screen the target appears. The validity effect (the outcome measure) is calculated by subtracting the reaction times to congruent from incongruent trials; a smaller validity effect indicates faster attentional switching on invalidly-cued trials and better task performance.

To assess correlations, Pearson's product-moment correlation coefficients were calculated (r and p values are reported in Tables 4 and 5). The data met all assumptions for the use of this test. Further details of the behavioural data are described elsewhere (14).

Figure 1. Representative volume masks for the regions that show significant genotype differences: (a) anterior cingulate, (b) cuneus, (c) parahippocampus. The white matter regions are coloured red and the grey matter regions are yellow. The slice positions for the sections are given in the figure.

III. RESULTS

A. Cuneus

White matter volume: Normalized white matter volume ratios in the cuneus were significantly greater in young adult e4+ ($T=4.2$, $p<0.001$), but there was no difference between genotype groups at mid age (Table 3). **Cortical thickness:** No significant genotype differences were observed in either age group. **Correlations with performance on covert attention task:** In young adults, there was a significant positive correlation between cuneus white matter volume and task performance in e4+, but not in e3/e3. At mid age, there was a trend to positive correlation in e3/e3 but not in e4+ (Table 4, Figure 2).

B. Anterior cingulate white matter

White matter volume: In the young adult group, white matter volume in the anterior cingulate was not significantly different between e4+ and e3/e3 for either age group. This is surprising given that previous findings have revealed the anterior cingulate is affected by APOE status. In light of this, and a recent perfusion study (43) that showed genotype-driven differences were lateralized to the left anterior cingulate, we decided to extend our study to the left and right anterior cingulate separately. This revealed that the white matter volume of the left anterior cingulate among the mid-age cohort was greater in e4+ (Table 3), and trending towards significance ($T=2.6$, $p=0.014$). **Cortical thickness:** No significant genotype

Table II

PERFORMANCE ON COVERT ATTENTION TASK AND EPISODIC MEMORY TASK BY GENOTYPE. STANDARD DEVIATIONS ARE GIVEN IN PARENTHESES.

Age	Genotype	Covert Attention Task			Episodic Memory (recalled words, max 20)
		RT (ms) to validly cued trials	RT (ms) to invalidly cued trials	Validity effect (ms)	
Young	e4+	358(50)	387(58)	26(5.5)	9.0 (2.7)
	e3/e3	329(72)	371(67)	42 (5.4)	9.7 (2.9)
Mid	e4+	422(84)	476(108)	54(44)	6.9 (2.3)
	e3/e3	393(90)	457(78)	64 (25)	7.9 (2.1)

Table III

TABLE 3. MEAN CORTICAL THICKNESS AND WHITE MATTER VOLUME RATIOS FOR THE PARAHIPPOCAMPUS (LEFT+RIGHT HEMISPHERE), CUNEUS (LEFT+RIGHT HEMISPHERE), POSTERIOR CINGULATE (LEFT+RIGHT HEMISPHERE) AND ANTERIOR CINGULATE (LEFT AND RIGHT HEMISPHERE). STANDARD DEVIATIONS ARE GIVEN IN PARENTHESES. THE P-VALUES SHOWN IN BOLD ARE BELOW THE BONFERRONI SIGNIFICANCE THRESHOLD, THOSE IN BOLD-ITALICS INDICATE TRENDS ONLY.

Brain region	Young			Mid		
	e4+	e3/e3	p value	e4+	e3/e3	p value
<i>WM volume ratio (x10⁻³)</i>						
Parahippocampus	2.2 (0.3)	2.1 (0.3)	0.396	2.2 (1.5)	2.3 (0.2)	0.389
Cuneus	3.3 (0.3)	2.9 (0.4)	<0.001	3.2 (0.4)	3.4 (0.4)	0.205
Precuneus	12.6 (1.4)	12.1 (1.7)	0.31	12.9 (1.7)	12.6 (1.5)	0.588
Left anterior cingulate	1.8 (0.3)	1.8 (0.3)	0.668	1.9 (0.2)	1.8 (0.1)	0.014
Right anterior cingulate	2.0 (0.2)	1.9 (0.2)	0.85	2.1 (0.3)	2.0 (0.3)	0.611
Posterior Cingulate	5.4 (0.4)	5.4 (0.5)	0.508	5.6 (0.5)	5.6 (0.3)	0.937
<i>Cortical Thickness (mm)</i>						
Parahippocampus	3.1 (0.2)	3.1 (0.3)	0.698	3.0 (0.1)	2.8 (0.2)	0.006
Cuneus	1.9 (0.1)	1.8 (0.1)	0.454	1.8 (0.1)	1.8 (0.1)	0.358
Precuneus	2.6 (0.2)	2.5 (0.1)	0.177	2.4 (0.1)	2.4 (0.1)	0.659
Left Anterior Cingulate	2.7 (0.2)	2.7 (0.2)	0.938	2.5 (0.1)	2.6 (0.1)	0.244
Right Anterior Cingulate	2.7 (0.2)	2.7 (0.2)	0.719	2.5 (0.2)	2.5 (0.1)	0.215
Posterior Cingulate	2.8 (0.2)	2.9 (0.2)	0.522	2.6 (0.1)	2.7(0.1)	0.473
Hippocampus*	5.6 (0.3)	5.6 (0.6)	0.634	5.6 (0.5)	5.7 (0.4)	0.309

* hippocampus grey matter volume ratio (x10⁻³).

Table IV

CORRELATIONS BETWEEN TASK PERFORMANCE AND WHITE MATTER VOLUME MEASURES. THE P-VALUES SHOWN IN BOLD ARE BELOW THE BONFERRONI-CORRECTED SIGNIFICANCE THRESHOLD.

Age	Genotype	Cuneus volume and validity effect	Left anterior cingulate volume and validity effect	Parahippocampal volume and episodic memory
Young	e4+	r = -0.63, p = 0.005	r = -0.595, p = 0.009	r = -0.286, p = 0.236
	e3/e3	r = -0.165, p = 0.501	r = 0.167, p = 0.494	r = -0.220, p = 0.380
Mid	e4+	r = 0.220, p = 0.395	r = -0.466, p = 0.059	r = 0.126, p = 0.631
	e3/e3	r = -0.545, p = 0.029	r = -0.115, p = 0.671	r = -0.02, p = 0.939

Table V

CORRELATIONS BETWEEN TASK PERFORMANCE AND CORTICAL THICKNESS MEASURES. THE P-VALUES SHOWN IN BOLD ARE BELOW THE BONFERRONI-CORRECTED SIGNIFICANCE THRESHOLD.

Age	Genotype	Cuneus thickness and validity effect	Left anterior cingulate thickness and validity effect	Parahippocampal thickness and episodic memory
Young	e4+	r = -0.047, p = 0.852	r = -0.099, p = 0.697	r = 0.590, p = 0.008
	e3/e3	r = -0.218, p = 0.370	r = -0.542, p = 0.017	r = 0.048, p = 0.846
Mid	e4+	r = 0.388, p = 0.124	r = -0.134, p = 0.608	r = 0.307, p = 0.231
	e3/e3	r = 0.154, p = 0.568	r = -0.109, p = 0.687	r = 0.044, p = 0.861

differences were observed in either age group. Correlations with performance on covert attention task: There was a significant positive correlation between task performance and normalized left anterior cingulate white matter volume in young adult e4+ only (Table 4, Figure 2).

C. Parahippocampus

White matter volume: No significant genotype differences in white matter volume were detected at either age group. Cortical thickness: In the young adult group, there were no significant genotype-dependent differences in parahippocampal thickness. At mid age, parahippocampal thickness was

significantly greater in e4+ (T=2.9, p=0.006; Table 3). Correlations with performance on episodic memory task: Number of words recalled was positively correlated with parahippocampal thickness in young adult e4+ only (Table 5, Figure 2).

D. Posterior cingulate and precuneus

White matter volume: No significant genotype differences in the posterior cingulate or precuneus were observed between genotypes for both age groups. Cortical thickness: No significant genotype differences were observed in either age group. Correlations with performance on covert attention task: There were no correlations between the MRI measures and task performance for either region.

Figure 2. Plots for the statistically-significant correlations ($p < 0.05$) of MR-derived white matter volume or cortical thickness with performance in covert attention or episodic memory tasks.

E. Hippocampus

Grey matter volume ratio: No significant genotype differences in the hippocampal grey matter were detected for both age groups. Correlations with performance on episodic memory task: There were no correlations between grey matter volume ratio and task performance.

F. Resting State MRI

1) *Young group*: From the results of the ICA, 8 resting state networks were identified. These were the default mode Network (DMN), Executive Control Network (ECN), left fronto-parietal, right fronto-parietal, medial visual network, lateral visual network, sensorimotor network, auditory network.

Voxel-wise comparisons of e4+ vs e3/e3 revealed differences within the medial visual network only. It was found that e3/e3 showed greater functional connectivity within a portion of this network, localised to extrastriate cortex ($x = 6$, $y = -82$, $z = -12$, FWE-corrected $p < 0.05$) (Figure 3).

2) *Mid-age group*: The same 8 resting state networks were identified for the mid-age group as those found in the young group. Voxel-wise comparisons revealed no APOE effects within any of the networks.

IV. DISCUSSION

This study has revealed subtle, but significant, structural differences between e3/e3 and e4+ at both young and mid-age that complement our finding of genotype-driven microstructural differences in our young cohort, published previously (59). The current work extends this earlier analysis in studying both young and mid-age cohorts. The previous analysis identified a subtle increase in white matter volume of e4+ in young group, so this work has targeted specific brain regions which have allowed us to identify regional volume and cortical thickness differences in two age groups that could not be detected by a whole-brain analysis. In addition, this present study focuses on the relationship between task performance and the structural differences in brain regions known to be implicated in completion of these tasks. We found that there were no detectable genotype-dependent differences in hippocampal volume for either the young or mid-aged adults in this study. This is not surprising given the somewhat conflicting findings

Figure 3. (a) The medial visual resting state network identified by ICA in the young adult group (b) young adult e3/e3 showed greater functional connectivity within this network as compared to e4+. The slice positions x and y are shown on the figure.

in the literature. A recent study (45) reported that hippocampal volume was greater in e4+ compared to e4-, but it should be noted that the study was conducted on a cohort with a wide age range (26-46 years) and with an average age (38 years) that bisects our two groups. Further studies were in keeping with our current finding that hippocampal volume does not differ by APOE status (26) but did correlate with episodic memory performance in e4+ in combination with other risk alleles (22). In contrast, another study in healthy individuals aged 50-80 found that e4+ have significantly reduced hippocampal volume and a positive correlation between recognition memory and hippocampal volume (68). The nearby parahippocampus is also an important region for memory processing. In this area we found that there was a significant positive correlation between the thickness of the parahippocampus in young e4+ and their performance on an episodic memory task, suggesting that behavioural benefits may be available for e4+ with increased thickness. However, there was no evidence that young adult e4+ had greater parahippocampal thickness overall and nor did they perform better as a group in the episodic memory task. In contrast, mid-age e4+ in our cohort did not show a correlation between parahippocampal thickness and task performance, but nevertheless possessed significantly greater overall parahippocampal thickness, bilaterally. The left anterior cingulate also displayed the same pattern: a positive correlation between white matter volume and task performance (this time in the covert attention task) was seen in young e4+ only, and increased white-matter volume was present in mid-age e4+. The laterality of our finding in the anterior cingulate mirrors that of a recent CBF study in APOE, where significant genotype differences were restricted to the left-sided anterior cingulate only (43).

The APOE gene codes for a protein that influences neuronal development in general, through its role in the transport of

cholesterol and lipid to structures such as the myelin sheath (69,70). In particular, mouse studies have demonstrated that the presence of APOE is crucial for neurogenesis (71,72) and that the process is dependent on the interaction of APOE isoform and environmental conditions. Several studies on human brains (73,74) have identified that neurodegeneration in diseases such as Huntington's and AD is actually accompanied by a compensatory increase in neurogenesis, at least in the early phase of the disease. In the case of AD, the increased volume observed in the hippocampus is not maintained but becomes atrophic as the disease progresses. Likely candidates for this eventual atrophy are increased cell loss coupled with impaired neural development in the potentially toxic environment of the AD brain (74). Our study observed significantly greater parahippocampal thickness and a trend towards greater left anterior cingulate white matter volume in our healthy e4+ at mid-age. Studies have shown that e4+ exhibit a significant increase in activity within the hippocampus across the lifespan (35,75-77) possibly as a compensatory up-regulation driven by reduced synaptic plasticity among this genetic group (78). From a neurogenic perspective, this greater hippocampal burden throughout life could promote enhanced neurogenesis in this region. The presence of a neuronal bottleneck in our e4+ could be one interpretation for the findings of the present study, where our correlations show that if young e4+ have thinner parahippocampal cortex or smaller white matter volume in the left anterior cingulate, they exhibit reduced performance in their respective tasks. Such bottlenecks have been demonstrated in multitasking trials where brain areas such as the hippocampus and anterior cingulate are overactivated (79). Increased cognitive load, in rodent studies at least, has given rise to increased neurogenesis at the narrowest region in the hippocampus (80). It is an intriguing possibility that an over-engagement with parahippocampus and anterior cingulate by e4+ throughout youth may provoke a neurogenic response that leads to the relative enlargement in these areas, as observed in our mid-age e4+ cohort. This pattern of non-linear cerebral atrophy has been reported in a recent study across several key brain areas, including the medial temporal structures (81). In that study, a thicker parahippocampus cortex among the e4+ was observed in the prodromal AD cohort, but the increased cortical thickness was not maintained and, mirroring previous findings in the hippocampus (73,74), it later reverted at later stages of the disease profile. Activity-driven changes to brain structure are not limited to grey matter; there is evidence for changes to axonal diameter, number of myelinated axons, and axonal sprouting in white matter. Such changes have been identified in the hippocampus (82) and also in the long-range axons (83) in response to learning, exercise, or recovery from damage. However, we present here a cross-sectional study: the young and mid-age cohorts contain different participants with varying social and educational backgrounds, so we are cautious not to draw conclusions that can only be satisfactorily made from longitudinal studies.

Our data also show several differences in the cuneus (extra-atriate) regions between our genotype groups. Again, there is a positive correlation between task performance (smaller validity effect in CA task) and cuneus white matter volume.

In contrast to the results discussed above, however, there is a significantly greater cuneus white matter volume in e4+ compared to e3/e3 at young age. These findings are also accompanied by the observation that the medial visual network was found to be underactivated in young e4+ compared to e3/e3 in just this region in resting state MRI. Various other studies have investigated APOE effects in the resting state: enhanced default mode network (DMN) coactivations have been reported in e4+ both at age 20-35 (35) and 50-80 (37). It has been shown that synchronization between hippocampus and DMN is elevated in mid-age and elderly e4+ and this correlates with memory performance; increased synchronization was also reported in other regions including the cuneus (37). Disrupted activity in DMN and executive control networks has also been reported in mid-aged (45-65) e4+, again linked to episodic memory performance (60) although we could find no differences in DMN coactivity in either of the age groups under study. Other work has also failed to replicate these findings (36,84), suggesting that the exact age range and/or other factors might be critical. Instead, we localized APOE differences to coactivation within the medial visual resting state network. Although we are the first study to report such a finding, this result supports the argument that the cuneus could be an important locus for APOE-related differences in structure and function. Visual resting state differences were found only in the young adult group; at mid-age, no APOE effects on resting state networks were found. Interpreting this result is difficult, not least because the significance of resting state connectivity in early visual processing areas is not well characterized. Importantly, however, the resting state data presented here, which found no differences in DMN coactivation by genotype, suggests that differences reported by (60) could have resulted from the inclusion of older adults in their sample. The current findings suggest that DMN coactivity does not differentiate e4+ and e3/e3 until at least age 55. Alternatively, lifestyle factors and educational attainment could be critical; further work is needed to investigate these.

This study has several limitations. Importantly, this is a cross-sectional study that recruited healthy participants with a range of educational and social backgrounds across the age groups and, therefore, a direct study of age and genotype interactions cannot be performed. Nevertheless, our findings raise some important questions about the interaction of brain function and structure across APOE genotypes that can only be resolved by future longitudinal studies. A further limitation is use of the Freesurfer software package, which does not always provide accurate estimates of the surfaces of the pial and grey matter in the temporal lobes of the brain. It is important, therefore, that this study recruited compliant, healthy volunteers that provided good quality images, requiring limited minor edits to the automated segmentation. Also, unreliable segmentation would reduce the precision of thickness and volume measures in all participants, without bias towards either genotype group. This would result in a reduced sensitivity to identifying group differences (i.e. more false negatives) and, in turn, true differences would need to be large or consistent across the group to be statistically significant. Finally, although we had a good sample size for this study, we did not include

either a homozygous APOE-e4 group, or an e2 group; the very low prevalence of these genotype configurations in the natural population requires a larger study and much wider genotype screening.

V. CONCLUSION

APOE status is known to affect the function of the human brain throughout the lifespan and many functional and resting state MRI studies have revealed differences between e4+ and e4-. The cuneus seems to be one of the first sites where amyloid is deposited in AD and, in this work, we have found that the cuneus appears to be an important locus for genotype differences with greater functional connectivity among the young e3/e3 and greater white matter volume in young e4+. In addition, we have correlated functional and behavioural differences with brain structure and found that subtle cortical thickness measures in the parahippocampus of our young e4+ positively correlate with performance on a memory task. A similar correlation with white matter volume was found in the left anterior cingulate on a covert attention task, suggesting that behavioural benefits may be available for e4+ with either increased cortical thickness or volume in these regions. In our mid-age cohort we see significant structural differences in these same regions: the parahippocampal cortical thickness and left anterior cingulate white matter volumes were both significantly greater for e4+ compared to e3/e3, offering the intriguing possibility that the greater engagement with these regions in youth may lead to observable neurogenic structural changes by mid age. These changes are not associated with behavioural benefits in younger adults, and may underwrite the absence of behavioural deficits in e4+ at mid age.

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REFERENCES

1. Prince M, Wimo A, Guerchet M, Ali G-C, Wu Y-T, Prina M. World Alzheimer Report 2015: The Global Impact of Dementia. London: Alzheimers Disease International; 2015.
2. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993;261(5123):921-923.
3. Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ, Hulette C, Crain B, Goldgaber D, Roses AD. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993;43(8):1467-1472.
4. Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. *Neuron* 2009;63(3):287-303.
5. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;240(4852):622-630.
6. Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including

Alzheimer's disease. *Proc Natl Acad Sci U S A* 2006;103(15):5644-5651.

7. Conejero-Goldberg C, Gomar JJ, Bobes-Bascaran T, Hyde TM, Kleinman JE, Herman MM, Chen S, Davies P, Goldberg TE. APOE2 enhances neuroprotection against Alzheimer's disease through multiple molecular mechanisms. *Molecular psychiatry* 2014;19(11):1243-1250.
8. Small BJ, Rosnick CB, Fratiglioni L, Backman L. Apolipoprotein E and cognitive performance: a meta-analysis. *Psychol Aging* 2004;19(4):592-600.
9. Wisdom NM, Callahan JL, Hawkins KA. The effects of apolipoprotein E on non-impaired cognitive functioning: a meta-analysis. *Neurobiology of aging* 2011;32(1):63-74.
10. Schiepers OJ, Harris SE, Gow AJ, Pattie A, Brett CE, Starr JM, Deary IJ. APOE E4 status predicts age-related cognitive decline in the ninth decade: longitudinal follow-up of the Lothian Birth Cohort 1921. *Molecular psychiatry* 2012;17(3):315-324.
11. Deary IJ, Whiteman MC, Pattie A, Starr JM, Hayward C, Wright AF, Carothers A, Whalley LJ. Cognitive change and the APOE epsilon 4 allele. *Nature* 2002;418(6901):932.
12. Marchant NL, King SL, Tabet N, Rusted JM. Positive effects of cholinergic stimulation favor young APOE epsilon4 carriers. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2010;35(5):1090-1096.
13. Mondadori CR, de Quervain DJ, Buchmann A, Mustovic H, Wollmer MA, Schmidt CF, Boesiger P, Hock C, Nitsch RM, Papassotiropoulos A, Henke K. Better memory and neural efficiency in young apolipoprotein E epsilon4 carriers. *Cereb Cortex* 2007;17(8):1934-1947.
14. Rusted JM, Evans SL, King SL, Dowell N, Tabet N, Tofts PS. APOE e4 polymorphism in young adults is associated with improved attention and indexed by distinct neural signatures. *NeuroImage* 2013;65:364-373.
15. Han SD, Bondi MW. Revision of the apolipoprotein E compensatory mechanism recruitment hypothesis. *Alzheimers Dement* 2008;4(4):251-254.
16. Tuminello ER, Han SD. The Apolipoprotein E Antagonistic Pleiotropy Hypothesis: Review and Recommendations. *International Journal of Alzheimer's Disease*. Volume 2011; 2011. p 1-12.
17. Rusted J, Carare RO. Are the effects of APOE 4 on cognitive function in nonclinical populations age- and gender-dependent? *Neurodegenerative disease management* 2015;5(1):37-48.
18. Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol* 1999;45(3):358-368.
19. den Heijer T, Oudkerk M, Launer LJ, van Duijn CM, Hofman A, Breteler MM. Hippocampal, amygdalar, and global brain atrophy in different apolipoprotein E genotypes. *Neurology* 2002;59(5):746-748.
20. Jak AJ, Houston WS, Nagel BJ, Corey-Bloom J, Bondi MW. Differential cross-sectional and longitudinal impact of APOE genotype on hippocampal volumes in nondemented older adults. *Dement Geriatr Cogn Disord* 2007;23(6):382-389.
21. Lemaitre H, Crivello F, Dufouil C, Gratiot B, Tzourio C, Alperovitch A, Mazoyer B. No epsilon4 gene dose effect on hippocampal atrophy in a large MRI database of healthy elderly subjects. *NeuroImage* 2005;24(4):1205-1213.
22. Ferencz B, Laukka EJ, Lovden M, Kalpouzos G, Keller L, Graff C, Wahlund LO, Fratiglioni L, Backman L. The influence of APOE and TOMM40 polymorphisms on hippocampal volume and episodic memory in old age. *Front Hum Neurosci* 2013;7:198.
23. Lyall DM, Royle NA, Harris SE, Bastin ME, Maniega SM, Murray C, Lutz MW, Saunders AM, Roses AD, del Valdes Hernandez MC, Starr JM, Porteous DJ, Wardlaw JM, Deary IJ. Alzheimer's disease susceptibility genes APOE and TOMM40, and hippocampal volumes in the Lothian birth cohort 1936. *PloS one* 2013;8(11):e80513.
24. O'Dwyer L, Lamberton F, Matura S, Tanner C, Scheibe M, Miller J, Rujescu D, Prvulovic D, Hampel H. Reduced hippocampal

volume in healthy young ApoE4 carriers: an MRI study. *PLoS one* 2012;7(11):e48895.

25. Shaw P, Lerch JP, Pruessner JC, Taylor KN, Rose AB, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN. Cortical morphology in children and adolescents with different apolipoprotein E gene polymorphisms: an observational study. *Lancet Neurol* 2007;6(6):494-500.

26. Khan W, Giampietro V, Ginesteta C, Dell'Acqua F, Bouls D, Newhouse S, Dobson R, Banaschewski T, Barker GJ, Bokde ALW, Buchel C, Conrod P, Flor H, Frouin V, Garavan H, Gowland P, Heinz A, Ittermann B, Lemaitre H, Nees F, Paus T, Pausova Z, Rietschel M, Smolka MN, Stroble A, Gallinat J, Westman E, Schumann G, Lovestone S, Simmons A. No Differences in Hippocampal Volume between Carriers and Non-Carriers of the ApoE epsilon 4 and epsilon 2 Alleles in Young Healthy Adolescents. *Journal of Alzheimers Disease* 2014;40(1):37-43.

27. Burggren AC, Zeineh MM, Ekstrom AD, Braskie MN, Thompson PM, Small GW, Bookheimer SY. Reduced cortical thickness in hippocampal subregions among cognitively normal apolipoprotein E epsilon 4 carriers. *NeuroImage* 2008;41(4):1177-1183.

28. Zhang S, Chen Y, Liu Z, Zhang J, Li X, Cui R, Zhang Z. Association of White Matter Integrity and Cognitive Functions in Chinese Non-Demented Elderly with the APOE epsilon 4 Allele. *J Alzheimers Dis* 2015;48(3):781-791.

29. Ito K, Sasaki M, Takahashi J, Uwano I, Yamashita F, Higuchi S, Goodwin J, Harada T, Kudo K, Terayama Y. Detection of early changes in the parahippocampal and posterior cingulum bundles during mild cognitive impairment by using high-resolution multi-parametric diffusion tensor imaging. *Psychiatry research* 2015;231(3):346-352.

30. Remy F, Vayssiere N, Saint-Aubert L, Barbeau E, Pariente J. White matter disruption at the prodromal stage of Alzheimer's disease: relationships with hippocampal atrophy and episodic memory performance. *NeuroImage* 2015;7:482-492.

31. Salat DH, Greve DN, Pacheco JL, Quinn BT, Helmer KG, Buckner RL, Fischl B. Regional white matter volume differences in nondemented aging and Alzheimer's disease. *NeuroImage* 2009;44(4):1247-1258.

32. Killiany RJ, Hyman BT, Gomez-Isla T, Moss MB, Kikinis R, Jolesz F, Tanzi R, Jones K, Albert MS. MRI measures of entorhinal cortex vs hippocampus in preclinical AD. *Neurology* 2002;58(8):1188-1196.

33. Chen Y, Li P, Gu B, Liu Z, Li X, Evans AC, Gong G, Zhang Z. The effects of an APOE promoter polymorphism on human cortical morphology during nondemented aging. *J Neurosci* 2015;35(4):1423-1431.

34. Song H, Long H, Zuo X, Yu C, Liu B, Wang Z, Wang Q, Wang F, Han Y, Jia J. APOE Effects on Default Mode Network in Chinese Cognitive Normal Elderly: Relationship with Clinical Cognitive Performance. *PLoS one* 2015;10(7):e0133179.

35. Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE. Distinct patterns of brain activity in young carriers of the APOE-epsilon 4 allele. *Proc Natl Acad Sci U S A* 2009;106(17):7209-7214.

36. Trachtenberg AJ, Filippini N, Ebmeier KP, Smith SM, Karpe F, Mackay CE. The effects of APOE on the functional architecture of the resting brain. *NeuroImage* 2012;59(1):565-572.

37. Westlye ET, Lundervold A, Rootwelt H, Lundervold AJ, Westlye LT. Increased hippocampal default mode synchronization during rest in middle-aged and elderly APOE epsilon 4 carriers: relationships with memory performance. *J Neurosci* 2011;31(21):7775-7783.

38. Mondadori CRA, Quervain DJ-FD, Buchmann A, Mustovic H, Wollmer A, Schmidt CF, Boesiger P, Hock C, Nitsch RM, Papassotiropoulos A, Henke K. Better Memory and Neural Efficiency in Young Apolipoprotein E epsilon 4 Carriers. *Cerebral Cortex* 2007;17(8):1934-1947.

39. Trachtenberg AJ, Filippini N, Cheeseman J, Duff EP, Neville MJ, Ebmeier KP, Karpe F, Mackay CE. The effects of APOE on brain activity do not simply reflect the risk of Alzheimer's disease. *Neurobiology of aging* 2012;33(3):618 e611-618 e613.

40. Fleisher AS, Sherzai A, Taylor C, Langbaum JB, Chen K, Buxton RB. Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. *NeuroImage* 2009;47(4):1678-1690.

41. Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *J Neurosci* 2005;25(34):7709-7717.

42. Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proc Natl Acad Sci U S A* 2004;101(13):4637-4642.

43. Wierenga CE, Clark LR, Dev SI, Shin DD, Jurick SM, Rissman RA, Liu TT, Bondi MW. Interaction of age and APOE genotype on cerebral blood flow at rest. *J Alzheimers Dis* 2013;34(4):921-935.

44. Lind J, Persson J, Ingvar M, Larsson A, Cruts M, Van Broeckhoven C, Adolfsson R, Backman L, Nilsson LG, Petersson KM, Nyberg L. Reduced functional brain activity response in cognitively intact apolipoprotein E epsilon 4 carriers. *Brain* 2006;129(Pt 5):1240-1248.

45. Alexander GE, Bergfield KL, Chen K, Reiman EM, Hanson KD, Lin L, Bandy D, Caselli RJ, Moeller JR. Gray matter network associated with risk for Alzheimer's disease in young to middle-aged adults. *Neurobiology of aging* 2012;33(12):2723-2732.

46. Protas HD, Chen K, Langbaum JB, Fleisher AS, Alexander GE, Lee W, Bandy D, de Leon MJ, Mosconi L, Buckley S, Truran-Sacrey D, Schuff N, Weiner MW, Caselli RJ, Reiman EM. Posterior cingulate glucose metabolism, hippocampal glucose metabolism, and hippocampal volume in cognitively normal, late-middle-aged persons at 3 levels of genetic risk for Alzheimer disease. *JAMA Neurol* 2013;70(3):320-325.

47. Dennis NA, Browndyke JN, Stokes J, Need A, Burke JR, Welsh-Bohmer KA, Cabeza R. Temporal lobe functional activity and connectivity in young adult APOE epsilon 4 carriers. *Alzheimers Dement* 2009.

48. Evans S, Dowell NG, Tabet N, Tofts PS, King SL, Rustad JM. Cognitive and neural signatures of the APOE epsilon 4 allele in mid-aged adults. *Neurobiology of aging* 2014;35(7):1615-1623.

49. Cabeza R, Daselaar SM, Dolcos F, Prince SE, Budde M, Nyberg L. Task-independent and task-specific age effects on brain activity during working memory, visual attention and episodic retrieval. *Cerebral Cortex* 2004;14(4):364-375.

50. Madden DJ, Spaniol J, Whiting WL, Bucur B, Provenzale JM, Cabeza R, White LE, Huettel SA. Adult age differences in the functional neuroanatomy of visual attention: A combined fMRI and DTI study. *Neurobiology of Aging* 2007;28(3):459-476.

51. Cho H, Seo SW, Kim JH, Suh MK, Lee JH, Choe YS, Lee KH, Kim JS, Kim GH, Noh Y, Ye BS, Kim HJ, Yoon CW, Chin J, Na DL. Amyloid Deposition in Early Onset versus Late Onset Alzheimer's Disease. *Journal of Alzheimers Disease* 2013;35(4):813-821.

52. Risacher SL, Kim S, Shen L, Nho K, Foroud T, Green RC, Petersen RC, Jack CR, Jr., Aisen PS, Koeppe RA, Jagust WJ, Shaw LM, Trojanowski JQ, Weiner MW, Saykin AJ. The role of apolipoprotein E (APOE) genotype in early mild cognitive impairment (E-MCI). *Frontiers in aging neuroscience* 2013;5:11.

53. Seo EH, Lee DY, Lee JM, Park JS, Sohn BK, Choe YM, Byun MS, Choi HJ, Woo JI. Influence of APOE genotype on whole-brain functional networks in cognitively normal elderly. *PLoS one* 2013;8(12):e83205.

54. Sheline YI, Morris JC, Snyder AZ, Price JL, Yan Z, D'Angelo G, Liu C, Dixit S, Benzinger T, Fagan A, Goate A, Mintun MA. APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF A-beta42. *J Neurosci* 2010;30(50):17035-17040.

55. Espeseth T, Westlye LT, Walhovd KB, Fjell AM, Endestad T, Rootwelt H, Reinvang I. Apolipoprotein E epsilon 4-related thickening of the cerebral cortex modulates selective attention. *Neurobiology of aging* 2012;33(2):304-322 e301.

56. Espeseth T, Greenwood PM, Reinvang I, Fjell AM, Walhovd KB, Westlye LT, Wehling E, Lundervold A, Rootwelt H, Parasuraman R. Interactive effects of APOE and CHRNA4 on attention and white matter volume in healthy middle-aged and older adults. *Cogn Affect Behav Neurosci* 2006;6(1):31-43.
57. Zatorre RJ, Fields RD, Johansen-Berg H. Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nature neuroscience* 2012;15(4):528-536.
58. Paez-Gonzalez P, Asrican B, Rodriguez E, Kuo CT. Identification of distinct ChAT(+) neurons and activity-dependent control of postnatal SVZ neurogenesis. *Nature neuroscience* 2014;17(7):934-942.
59. Dowell NG, Ruest T, Evans SL, King SL, Tabet N, Tofts PS, Rustad JM. MRI of carriers of the apolipoprotein E e4 allele-evidence for structural differences in normal-appearing brain tissue in e4+ relative to e4- young adults. *NMR Biomed* 2013;26(6):674-682.
60. Goveas JS, Xie C, Chen G, Li W, Ward BD, Franczak MB, Jones JL, Antuono PG, Li SJ. Functional network endophenotypes unravel the effects of apolipoprotein E epsilon 4 in middle-aged adults. *PloS one* 2013;8(2):e55902.
61. Rebeck GW, Reiter JS, Strickland DK, Hyman BT. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron* 1993;11(4):575-580.
62. Reuter M, Rosas HD, Fischl B. Highly accurate inverse consistent registration: A robust approach. *Neuroimage* 2010;53(4):1181-1196.
63. Segonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B. A hybrid approach to the skull stripping problem in MRI. *Neuroimage* 2004;22(3):1060-1075.
64. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM. Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002;33(3):341-355.
65. Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D, Caviness V, Makris N, Rosen B, Dale AM. Automatically parcellating the human cerebral cortex. *Cerebral Cortex* 2004;14(1):11-22.
66. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Sciences of the United States of America* 2000;97(20):11050-11055.
67. Thiel CM, Zilles K, Fink GR. Cerebral correlates of alerting, orienting and reorienting of visuospatial attention: an event-related fMRI study. *NeuroImage* 2004;21(1):318-328.
68. Lind J, Larsson A, Persson J, Ingvar M, Nilsson LG, Backman L, Adolfsson R, Cruts M, Sleegers K, Van Broeckhoven C, Nyberg L. Reduced hippocampal volume in non-demented carriers of the apolipoprotein E epsilon4: relation to chronological age and recognition memory. *Neurosci Lett* 2006;396(1):23-27.
69. Bu G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nature reviews* 2009;10(5):333-344.
70. Reinvang I, Espeseth T, Westlye LT. APOE-related biomarker profiles in non-pathological aging and early phases of Alzheimer's disease. *Neuroscience and biobehavioral reviews* 2013;37(8):1322-1335.
71. Adeosun SO, Hou X, Zheng B, Stockmeier C, Ou X, Paul I, Mosley T, Weisgraber K, Wang JM. Cognitive deficits and disruption of neurogenesis in a mouse model of apolipoprotein E4 domain interaction. *The Journal of biological chemistry* 2014;289(5):2946-2959.
72. Yang CP, Gilley JA, Zhang G, Kernie SG. ApoE is required for maintenance of the dentate gyrus neural progenitor pool. *Development (Cambridge, England)* 2011;138(20):4351-4362.
73. Curtis MA, Penney EB, Pearson AG, van Roon-Mom WM, Butterworth NJ, Dragunow M, Connor B, Faull RL. Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. *Proc Natl Acad Sci U S A* 2003;100(15):9023-9027.
74. Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC, Greenberg DA. Increased hippocampal neurogenesis in Alzheimer's disease. *Proc Natl Acad Sci U S A* 2004;101(1):343-347.
75. Bondi MW, Houston WS, Eyler LT, Brown GG. fMRI evidence of compensatory mechanisms in older adults at genetic risk for Alzheimer disease. *Neurology* 2005;64(3):501-508.
76. Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, Small GW. Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med* 2000;343(7):450-456.
77. Fleisher AS, Houston WS, Eyler LT, Frye S, Jenkins C, Thal LJ, Bondi MW. Identification of Alzheimer disease risk by functional magnetic resonance imaging. *Archives of neurology* 2005;62(12):1881-1888.
78. Chen Y, Durakoglulugil MS, Xian X, Herz J. ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci U S A* 2010;107(26):12011-12016.
79. Dux PE, Ivanoff J, Asplund CL, Marois R. Isolation of a central bottleneck of information processing with time-resolved fMRI. *Neuron* 2006;52(6):1109-1120.
80. Kempermann G. Why new neurons? Possible functions for adult hippocampal neurogenesis. *J Neurosci* 2002;22(3):635-638.
81. Gispert JD, Rami L, Sanchez-Benavides G, Falcon C, Tucholka A, Rojas S, Molinuevo JL. Nonlinear cerebral atrophy patterns across the Alzheimer's disease continuum: impact of APOE4 genotype. *Neurobiology of aging* 2015;36(10):2687-2701.
82. Toscano-Silva M, Gomes da Silva S, Scorza FA, Bonvent JJ, Cavalheiro EA, Arida RM. Hippocampal mossy fiber sprouting induced by forced and voluntary physical exercise. *Physiology & behavior* 2010;101(2):302-308.
83. Johansen-Berg H. Structural plasticity: rewiring the brain. *Curr Biol* 2007;17(4):R141-144.
84. Koch W, Teipel S, Mueller S, Benninghoff J, Wagner M, Bokde AL, Hampel H, Coates U, Reiser M, Meindl T. Diagnostic power of default mode network resting state fMRI in the detection of Alzheimer's disease. *Neurobiology of aging* 2012;33(3):466-478.