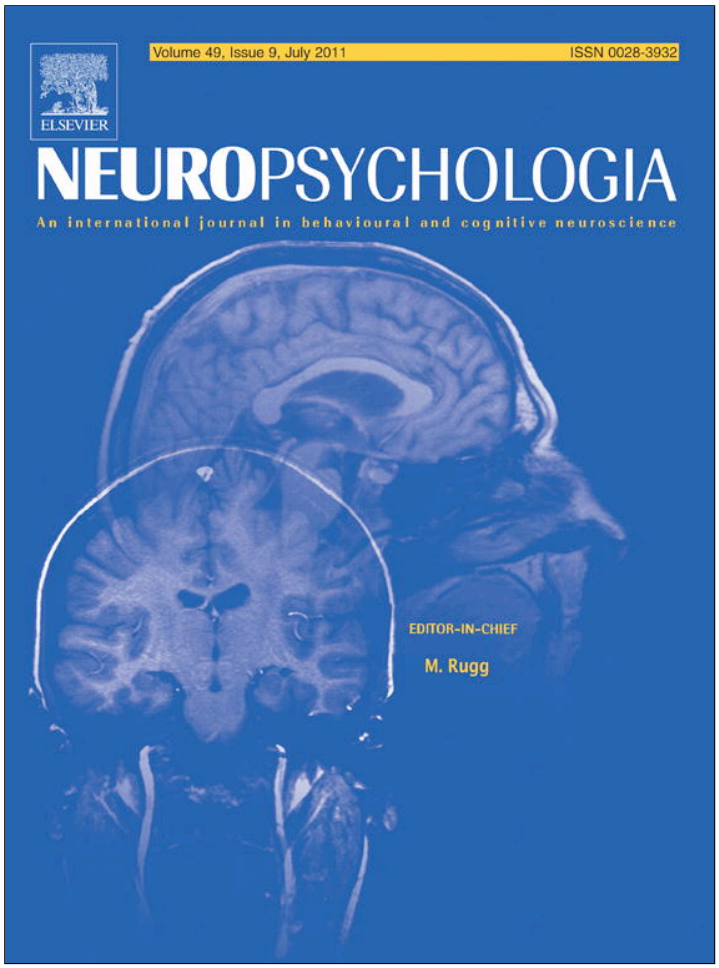

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Reduced hippocampal activity during encoding in cognitively normal adults carrying the APOE ε4 allele

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ABSTRACT

Apolipoprotein (APOE) ε4-related differences in memory performance have been detected before age 65. The hippocampus and the surrounding medial temporal lobe (MTL) structures are the first site affected by Alzheimer's disease (AD) and the MTL is the seat of episodic memory, including visuo-spatial memory. While reports of APOE ε4-related differences in these brain structures are not consistent in either cross-sectional or longitudinal structural and functional magnetic resonance imaging (fMRI) studies, there is increasing evidence that brain activity at baseline (defined as activity during fixation or rest) may differ in APOE ε4 carriers compared to non-carriers. In this fMRI study, cognitively normal APOE ε4 carriers and non-carriers engaged in a perspective-dependent spatial learning task (Shelton & Gabrieli, 2002) previously shown to activate MTL structures in older participants (Borghesani et al., 2008). A low-level, visually engaging dot-control task was used for comparison, in addition to fixation. APOE ε4 carriers showed less activation than non-carriers in the hippocampus proper during encoding. Specifically, when spatial encoding was contrasted against the dot-control task, encoding-related activation was significantly lower in carriers than non-carriers. By contrast, no ε4-related differences in the hippocampus were found when spatial encoding was compared with fixation. Lower activation, however, was not global since encoding-related activation in early visual cortex (left lingual gyrus) was not different between APOE ε4 carriers and non-carriers. The present data document APOE ε4-related differences in the hippocampus proper during encoding and underscore the role of low-level control contrasts for complex encoding tasks. These results have implications for fMRI studies that investigate the default-mode network (DMN) in middle-aged to older APOE ε4 carriers to help evaluate AD risk in this otherwise cognitively normal population.

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1. Introduction

Carriers of apolipoprotein (APOE) ε4 are at an increased risk of developing late onset Alzheimer's disease (AD) (Corder et al., 1993; Saunders et al., 1993; Tanzi & Bertram, 2001). Carrying at least one ε4 allele is a predictor of clinical progression from Mild Cognitive Impairment (MCI) to AD (de Leon et al., 2007; DeCarli et al., 2001; Landau et al., in press; Petersen et al., 1995). In cognitively normal populations, APOE ε4-related differences in neuropsychological task performance have been detected before age 65 (Adamson et al., 2010; Blair et al., 2005; Caselli et al., 2009; Kozauer, Mielke, Chan, Rebok, & Lyketsos, 2008;), although differences are typically modest (Small, Rosnick, Fratiglioni, & Backman, 2004; Wisdom, Callahan, & Hawkins, 2011). The medial temporal lobe (MTL) is the seat of episodic memory (Eichenbaum, 2000; Schacter & Wagner, 1999; Squire, Stark, & Clark, 2004), including visuo-spatial memory (Burgess, Maguire, & O'Keefe, 2002), and the first site affected by AD (Braak & Braak, 1997). However, reports of APOE ε4-related differences in brain structure, particularly in the MTL, are not consistent (Wierenga & Bondi, 2007). This is especially the case in cross-sectional studies, which have alternately revealed smaller and no difference in hippocampal volumes in APOE ε4 carriers compared to non-carriers (reviewed in Adamson et al., 2010). While it is possible that the impact of APOE ε4 on hippocampal volume changes over time will turn out to be larger or more consistent than single-time point assessments, more timely methods of assessing early indications of AD pathology are needed. Functional magnetic resonance imaging (fMRI) studies frequently focus on MTL subregions

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to capture activation patterns that are predictive of subsequent clinically significant decline (Bookheimer et al., 2000) and predictive of progression from MCI to AD (Machulda et al., 2003). Studying APOE ε4-related hippocampal and MTL cortical activity differences during an episodic memory task may prove promising for evaluating the risk of AD associated with APOE ε4 genotype in cognitively normal older adults.

Results from recent fMRI studies using episodic memory paradigms, however, have not been consistent in evaluating the APOE ε4 risk for AD in cognitively normal older adults (Cherbuin, Leach, Christensen, & Anstey, 2007; Trachtenberg, Filippini, & Mackay, in press). Several studies followed the approach of measuring brain activity relative to fixation or rest periods. While an increase in MTL Blood Oxygen Level Dependent (BOLD) activity was reported in APOE ε4 carriers using word-pair (Bookheimer et al., 2000; Fleisher et al., 2005) and verbal paired-associate tasks (Han et al., 2007), a decrease was reported in APOE ε4 carriers during spatial learning (Borghesani et al., 2008) and semantic categorization (Lind et al., 2006). No APOE ε4-related differences were reported during another paired-associate task (Bassett et al., 2006).

There is increasing evidence (Gusnard & Raichle, 2001; Stark & Squire, 2001) that 'baseline' fixation and rest conditions of functional imaging paradigms are associated with activation in the MTL. Studies that used rest or fixation conditions to examine the default mode network (which includes the MTL as a node) have reported abnormal activity in cognitively normal APOE ε4 carriers (Fleisher et al., 2009; Pihlajamaki & Sperling, 2009). Several fMRI studies examining possible APOE ε4-related differences during episodic memory encoding avoided the baseline issue by using 'tighter' or 'higher-level' contrasts, such as comparisons between the encoding of novel versus familiar stimuli. Three of these studies found reduced MTL activation in ε4 carriers compared to non-carriers during encoding of novel versus familiar words or pictorial stimuli (Borghesani et al., 2008; Lind et al., 2006; Trivedi et al., 2006). Two other studies found that the direction (increase or decrease) of differences in activation between APOE ε4 carriers and non-carriers in MTL during encoding of novel versus familiar items also depends on family history of AD (Johnson et al., 2006) and laterality (Bondi, Houston, Eyler, & Brown, 2005).

Han et al. (2007), on the other hand, have argued that comparing two high-level contrasts, such as novel and familiar conditions, may both entail encoding in the MTL making it difficult to isolate encoding processes relative to the task at hand (see also Buckner, Wheeler, & Sheridan, 2001). Despite this argument, using fixation as a low-level contrast is not an appropriate baseline state for assessing the underlying encoding processes involved in a complex cognitive task, because fixation has been repeatedly observed to activate the MTL (Stark & Squire, 2001). With these issues about high- versus low-level task contrasts in mind, the present study used a visually engaging dot-control task, in addition to fixation, to provide a low-level control that may provide a cleaner assessment of differences between healthy APOE ε4 carriers and non-carriers in MTL activation during encoding.

Recently, delpolyi, Rankin, Mucke, Miller, and Gorno-Tempini (2007) reported that atrophy in MTL regions involved in navigation – mainly the hippocampus, parahippocampus and retrosplenial cortex – is accompanied by navigation deficits in AD and MCI, suggesting that assessments of spatial memory can serve as an important marker for AD-related cognitive deficits. Topographic disorientation, or the feeling of being lost, is often reported as an early symptom of AD and worsens with disease progression (Pai & Jacobs, 2004). Two distinct methods – survey and route learning assays – have been previously employed to assess spatial learning and memory. Survey learning requires utilization of the relationships between landmarks and the environment, from a bird's eye view, resulting in acquisition of a global spatial representation. In contrast, route learning results in the building of a spatial representation from an egocentric ground-level perspective.

Extensive evidence implicates a putative 'spatial network', inclusive of the hippocampus, parahippocampal gyrus, posterior parietal, and retrosplenial regions, and interactions between these regions, in spatial learning (Moscovitch, Nadel, Winocur, Gilboa, & Rosenbaum, 2006; Seltzer & Pandya, 1984; Suzuki & Amaral, 1994). For example, extant evidence suggests that the right hippocampus, parahippocampal gyrus, posterior parietal cortex, and anterior temporal cortex play an important role in creating allocentric cognitive maps (Burgess et al., 2002; Gorno-Tempini & Price, 2001; McNamara & Shelton, 2003). The hippocampus is also implicated in building "cognitive maps" during spatial learning from a route perspective, such that information about objects and their location in the local environment is updated as one moves through space (Shelton & Gabrieli, 2002; Shelton & McNamara, 2004). It is important to note that the true nature of egocentric and allocentric systems varies on other dimensions as well, including whether one is in the space or external to it, and whether the global structure is more readily available. Nevertheless, the putative 'spatial network' is active during environment encoding via perspective-dependent learning tasks, with a subset of regions being active both when stimuli are encoded from a survey perspective and from a route perspective (Shelton & Gabrieli, 2002; Shelton & McNamara, 2004).

Recently, Borghesani et al. (2008) utilized a perspective-dependent learning task (Shelton & Gabrieli, 2002) to explore APOE ε4-related differences in brain activation during survey and route encoding. When compared with fixation, this study revealed reduced MTL activity during the encoding of both survey and route perspectives in APOE ε4 carriers. In addition, when compared with survey encoding, there was reduced MTL activity during route encoding in APOE ε4 carriers.

Building on these observations, the present study examined APOE ε4-related differences in hippocampal activity during a perspective-dependent learning task in older adults. This task has previously shown to activate the MTL robustly in young (Shelton & Gabrieli, 2002; Shelton & Pippitt, 2007) and older (Borghesani et al., 2008) adults. In addition to fixation, we employed a low-level, visually engaging dot-control task for comparison with the route and survey encoding tasks. The hypotheses were:

(1) APOE ε4 carriers will show reduced activation, relative to non-carriers, in the hippocampus proper during encoding of route and survey perspectives compared to the dot-control task. This is based on previous findings suggesting that APOE ε4 carriers may not activate hippocampus and the surrounding MTL structures to the extent that non-carriers do, perhaps due to functional and/or structural dysregulation that may indicate AD-related processes (Braak & Braak, 1997).
(2) Additionally, APOE ε4 carriers will show reduced activation, relative to non-carriers, in regions beyond the hippocampus proper that are involved in encoding (compared to the dot-control task). We focused on regions implicated in the default mode network (DMN), as reported in Pihlajamaki and Sperling (2009).

2. Materials and methods

2.1. Participants

A total of 26 cognitively normal adults (4 women and 22 men) were enrolled. Out of these participants, data from 5 participants were excluded from analysis: 3 due to movement greater than 4 mm, 1 due to a software problem, and 1 due to problems with hippocampal structural segmentation. Therefore, data from 21 participants (3 women and 18 men) were analyzed. Ten of the 21 adults were APOE ε3/4 carriers and the remaining 11 had the ε3/3 genotype. See Table 1 for demographics according to APOE ε4 status. All participants performed within normal limits on Rey AVLT delayed recall (range = 5–15/15 words recalled), Trail Making B (range = 32–107 s) and FAS

Table 1
Demographic characteristics of the 21 participants by APOE ε4.

	APOE ε4 non-carriers $n=11$	APOE ε4 carriers $n=10$
Age, y, mean ± SD	64.0 ± 5.1 (range 56–73)	63.5 ± 5.5 (range 54–70)
Education, y, mean ± SD[a]	16.7 ± 1.1	17.6 ± 2.1
Number White, non-Hispanic	9	9
Number men	8	10
Rey AVLT delayed, mean ± SD	10.4 ± 3.0	9.7 ± 3.0
Percentile range	(14.5–99.0)	(18.0–99.0)
Phonemic fluency, mean ± SD	37.8 ± 9.2	40.8 ± 10.9
Percentile range[b]	(9.5–85.0)	(9.5–75.0)
Trails B, mean ± SD	56.1 ± 23.2	64.7 ± 14.6
Percentile range	(14.5–99.0)	(34.5–92.0)
Hippocampal volume (cm^3)[c]	5.3 ± 0.6	5.1 ± 0.5
Number hypertension medication use ever	3	3
Number family history of dementia	3	6

[a] Education years greater than 20 years were truncated to 20 years.
[b] Phonemic fluency test (FAS) where participants are asked to generate as many words as possible that start with a specific letter of the alphabet in 60 s. A total score for 3 letters (FAS) is calculated.
[c] Hippocampal volume is residualized on Total Intracranial Volume (TIV) and then converted back to cc's for clarity.

phonemic fluency tests (range = 23–54 words), based on published age-based norms (Geffen, 1995; Ivnik et al., 1992; Ivnik, Malec, Smith, Tangalos, & Petersen, 1996; Tombaugh, Rees, & McIntyre, 1998; Tombaugh, Kozak, & Rees, 1996). Participants ranged in age from 54 to 73 years (mean = 63.75 ± 5.3). Only six participants were on hypertension medication.

These participants were selectively recruited from participants of the ongoing longitudinal Stanford/VA Aviation Study who had been previously genotyped for APOE. (Of 190 APOE-genotyped participants in the Aviation Study, 56.84% have the ε3/3 genotype, 22.1% have the ε3/4 genotype, 2.1% ε4/4, and the remaining have one or two ε2 alleles). Allele frequencies in the main aviator cohort were: ε2 = 10.3%, ε3 = 76.3% and ε4 = 13.4%. These allele frequencies are typical for nondemented Caucasian populations (Farrer et al., 1997). Entry criteria for the longitudinal Aviation Study are: age between 40 and 69 years, current FAA medical certificate, and currently flying with at least 300 but no more than 15,000 hours of total flight time. The majority of Aviation Study participants are recreational pilots, although a minority are flight instructors, air transport pilots, or have had job duties that include aircraft piloting or mechanics (see Taylor et al., 2007 for additional details). Informed consent, approved by Stanford University and VA Palo Alto Health Care System Institutional Review Boards, was obtained from each participant.

2.2. Procedures

2.2.1. DNA acquisition and APOE genotyping

APOE genotyping was performed with genomic DNA extracted from samples of frozen whole blood, buccal mucosa, or saliva. For blood samples, we used the Gentra PureGene kit (Gentra Systems, Minneapolis, MN); for buccal mucosa samples we used the protocol of Richards et al. (1993); and for saliva, we extracted DNA from epithelial cells using the Oragene kit (DNA Genotek, Ottowa, ON). APOE genotyping was performed as previously described (Murphy, Taylor, Kraemer, Yesavage, & Tinklenberg, 1997). Two independent observers who were blind to participants' neuroimaging data determined APOE genotypes, and all staff involved in testing or neuroimaging analysis were blind to the participants' genotypes.

2.2.2. Experimental task

Participants underwent an fMRI scan while performing a perspective-dependent spatial learning task originally designed by Shelton and colleagues (Shelton & Gabrieli, 2002; Shelton & McNamara, 2004; Shelton & Pippitt, 2007). Essentially, movies of two environments (park and market) were presented during the encoding practice and the encoding fMRI scanning session (for details on the environments, see Shelton & Gabrieli, 2002). The route movies for each environment were created from the perspective of a 6-foot-tall observer walking through the environment. The survey movies were created from the perspective of an aerial observer (70 ft above ground level in virtual space), looking straight down with 20% of the environment visible at any moment (see Fig. 1). Each participant viewed a route movie and a survey movie, with one complete viewing of each movie lasting 46 s; thus, each participant saw the same two movies throughout the encoding (e.g., market-route and park-survey were repeatedly viewed by a participant). Across participants, the two environments were presented from the route or survey perspectives. A 20-s long dot-control task, previously designed by Shelton and colleagues (unpublished), was presented along with the route and survey tasks in each block. During the dot-control task, nine consecutive red and blue dots were presented, one per 1 s, on a noisy background (equal luminosity to the route and survey movies). The participants were instructed to indicate the color of the last dot by pressing a corresponding key on the button box.

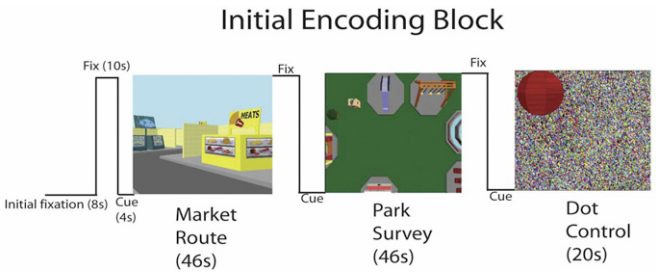

Fig. 1. The initial block of the perspective dependent encoding task. The initial fixation lasted 8 s in the beginning of the first block, followed by a fixation (10 s) and an environment cue (park or market). Block order was determined by using all orders of the movies (route, survey, and dot-control) repeated six times, with the constraint that no condition was repeated directly. During each scan, one environment was shown in the route perspective while the other was shown in the survey perspective. Two different orders were used to counterbalance which environment was seen first as route or survey.

As noted above, each participant was assigned to learn two environments, one as a route and one as a survey. Before scanning, participants viewed each environment and the dot-control task one time to familiarize themselves with tasks. During this initial viewing, the experimenter identified the landmarks in each environment by name as they were encountered. Participants were informed that they would not need to recall the landmark names per se, and thus their goal was to familiarize themselves with the location of the landmarks. Participants were asked to learn each environment as well as possible for a later memory test.

During the functional scan, route, survey and dot-control movies were repeated six times along with 10-s blocks of fixation before each movie. Order for the movies was determined by using all possible orders of conditions (route, survey and dot-control) across the six counterbalanced repetitions, with the constraint that there be no direct repetitions of any condition. During fixation blocks, participants were instructed to fixate on a white cross displayed in the middle of a black screen. Participants were assigned to one of four versions of the experiment where a different order was used to counterbalance which environment and which perspective was seen first. By chance, seven of the 11 APOE ε4 carriers saw the route perspective first, and seven of the ten non-carriers saw survey first. After scanning, to assess memory for the two environments, participants were asked to first draw a map of each environment from memory, and then place labeled color forms for the objects on a board depicting each environment.

2.2.3. FMRI data acquisition

Whole-brain imaging data were acquired on a 3 Tesla MRI system (General Electric Medical Systems). The scan session began with an in-plane anatomical scan, followed by a series of five 1.5-min scans for a word-list learning task (data not reported here), followed by the 15-min functional scan for the perspective-dependent spatial learning task. T2-weighted spin-echo anatomical images were acquired in 30 contiguous 6-mm coronal slices [30 ms TE; 4000 ms reaction time (TR)]. Functional images were acquired in the same slices using a T2*-sensitive gradient echo spiral pulse sequence (Glover & Lai, 1998) (30 ms TE; 2000 ms TR; 77° flip angle; 22 cm field of view; 64 × 64 acquisition matrix).

2.2.4. Image processing

Image processing was performed using SPM2 (Wellcome Department of Cognitive Neurology, London, UK). The first 4 volumes of each functional series were discarded to allow T1 equilibrium. All functional images were then corrected for participant motion. Each participant's structural images were coregistered to the mean functional image and segmented into grey matter, white matter and cerebrospinal fluid. Skull stripping was done using Brain Xtract tool in SPM99 on T1 images. The grey matter volume was normalized to the MNI grey matter template, and the normalization parameters were applied to all the structural and functional volumes. Functional images were spatially smoothed using a 8-mm FWHM Gaussian kernel.

Individual statistical models were constructed in SPM2 for each participant using the assumptions of a general linear model. Encoding phases of route, survey, and dot-control movies were modelled as blocks. Regressors were added to control for participant motion. Statistical contrasts were constructed to compare route vs dot-control, route vs. fixation, route vs. survey, survey vs. dot-control, survey vs. fixation, and dot-control vs. fixation. These contrasts were used to obtain participant-specific estimates for each effect that were then entered into a second-level analysis, with participant as a random effect. A one-sample *t*-test against a contrast value of zero was then performed at each voxel. Voxel-based group effects are first reported at $p < .001$, uncorrected and were considered significant if they a) exceeded a threshold of $p < .05$, False Discovery Rate (FDR) corrected, and b) consisted of five or more contiguous voxels.

To generate a hippocampal region-of-interest (ROI), an anatomical mask for the hippocampus was created using a high-resolution T1 structural MRI scan of each participant. These scans were acquired on a 1.5 T GE scanner approximately 2 yrs previously as part of the Stanford/VA aviator MRI study (Adamson et al., 2010). The time lag between the structural and functional scans were not confounded with the overall BOLD response (for route: $r = -.26$; $p > .1$; survey: $r = -.09$; $p > .1$; dot-control: $r = .1$; $p > .1$) or with APOE $\varepsilon 4$ status ($r = .06$; $p > .1$). Semi-automated hippocampal voluming was performed using a commercial high-dimensional brain-mapping tool (Medtronic Surgical Navigation Technologies, Louisville, CO; for details of these procedures, see (Csernansky et al., 2000; Hsu et al., 2002; Kramer et al., 2005)). Briefly, 22 control points were manually placed as local landmarks around each participant's hippocampus. These landmarks included one at the hippocampus head, one at the tail, and one each at the superior, inferior, medial and lateral boundaries of the hippocampus. Each participant's brain was matched to a template brain via fluid image transformation, and the pixels within the boundaries of the hippocampus were counted to estimate the volume. Experienced tracers confirmed the boundaries of the markings before computing the estimation of each final hippocampal volume.

This hippocampal anatomical mask was created for each participant and was co-registered with each participant's functional data. The parameter estimates were averaged across all voxels within each subjects' mask. Complementing the voxel-level analyses, the beta weights associated with particular contrasts were extracted from this hippocampal ROI. To compare the hippocampal activation with activity in a brain region not usually reported to be influenced by APOE $\varepsilon 4$ status, a control ROI was chosen *a priori*. This region was defined anatomically in the visual cortex based on the anatomical region (left lingual gyrus/calcarine fissure (peak voxel at −6, −81, 0, BA 17/18)) that was activated in both route and survey conditions (compared to fixation) in the original study (Shelton & Gabrieli, 2002). The beta estimates for all voxels within a 6-mm radius of the maximum from these coordinates were extracted. The beta estimates from the hippocampal and visual cortex ROIs for the route, survey and the dot-control conditions were submitted to analysis of variance, using SPSS 18 (Chicago, IL), with factors of task condition and APOE status.

3. Results

3.1. Demographics

Table 1 summarizes characteristics of the 21 participants who were included in the study, separated by APOE $\varepsilon 4$ carrier status. There were no significant differences between $\varepsilon 4$ carriers and non-carriers in demographic characteristics, Rey AVLT delayed recall performance, Trails B, FAS phonemic fluency tests or hippocampal volumes (all p's > .1).

3.2. Behavioral results

To assess participants' memory for the two environments, a point-rating scale was used to score the post-scan tasks of recognition (placement of landmark-labeled color forms) and recall (map drawings). Specifically, 1 point was given if the object placed or drawn was an object presented but was not in the correct location; 1 point was given if the object placed or drawn was incorrect but was placed in a location that previously contained an object; and, 2 points were given if the object placed or drawn was present and in the correct location. A summary score was calculated as the average number of points divided by the number of objects presented. Maximum possible score for recognition or recall was 2.

For recognition, there was no effect of Perspective (F (1, 19) = 2.11, $p > .1$), APOE Genotype (F (1, 19) = 0.37, $p > .1$), nor a Genotype x Perspective interaction (F (1, 19) = 0.38, $p > .1$). The total score for the color forms task for APOE $\varepsilon 4$ carriers was 1.17 ± 0.37 (park survey = 1.46 ± 0.30; park route = 1.11 ± 0.42; market survey = 1.07 ± 0.39; market route = 1.04 ± 0.33) and for APOE $\varepsilon 4$ non-carriers was 1.24 ± 0.26 (park survey = 1.41 ± 0.30; park route = 1.14 ± 0.30; market survey = 1.27 ± 0.11; market route = 1.11 ± 0.28). Similarly, for recall, there was no effect of Perspective (F (1, 19) = 3.50, $p > .1$), APOE Genotype (F (1, 19) = 0.52, $p > .1$) nor a Genotype × Perspective interaction (F (1, 19) = 0.52, $p > .1$). The total score for the map drawing task for APOE $\varepsilon 4$ carriers was 0.64 ± 0.26 (park survey = 0.55 ± 0.23; park route = 0.99 ± 0.29; market survey = 0.52 ± 0.08; market route = 0.52 ± 0.27) and for APOE $\varepsilon 4$ non-carriers was 0.72 ± 0.31 (park survey = 0.62 ± 0.22; park route = 0.88 ± 0.25; market survey = 0.62 ± 0.15; market route = 0.71 ± 0.50).

3.3. FMRI results

3.3.1. Activation differences in the hippocampus

Our primary hypothesis was that APOE $\varepsilon 4$ carriers will show reduced activation, relative to non-carriers, in the hippocampus during encoding of route and survey perspectives compared to dot-control. To test this, we analyzed the functional activity in an anatomically defined hippocampal ROI. Fig. 2a illustrates the mean estimated BOLD response in $\varepsilon 4$ carriers and non-carriers during the survey encoding, route encoding, and dot-control tasks (relative to fixation). To understand how the three task conditions and APOE status influenced hippocampal activation, we ran a mixed-model ANOVA, with task as a within-subject factor and genotype as a between-subjects factor. Two types of planned comparisons were used to examine differences among the three tasks and to parse task × APOE status interactions: (1) comparisons related to spatial encoding (average of route and survey vs. dot-control), and (2) comparisons related to the effect of perspective (route vs. survey).

Task-related activation differences: The main effect of task was significant (F (2, 38) = 11.64, $p < .001$). The first planned comparison revealed that the two spatial encoding tasks showed a trend for eliciting greater activation relative to the dot-control task (F (1, 38) = 3.65, $.05 < p < .1$). The second planned comparison revealed that the difference between the route and survey perspectives was numerically in the direction observed in previous studies (route > survey), but it did not approach significance in this sample, (F (1, 38) = 0.48, $p > .1$; Fig. 2a).

APOE $\varepsilon 4$-related activation differences: There was no main effect of genotype (F (1, 19) = .26, $p > .1$). However, the hypothesized interaction between genotype and task was significant (F (2, 38) = 5.29, $p = .009$; Fig. 2a). This interaction reflected reduced encoding-related activation (average of route and survey vs. dot-control) for the carriers compared to the non-carriers (F (1, 38) = 19.2, $p < .001$). In fact, in the carriers there was no significant difference between the route and survey tasks vs. dot-control ($t = 1.27$, $p > 0.1$), whereas in the non-carriers there was a significant increase in activation during route and survey vs. dot-control ($t = 4.76$, $p < 0.001$). When analysis was restricted to the contrast of the two spatial encoding tasks, there was no difference between $\varepsilon 4$ carriers and non-carriers (F (1, 38) = 0.01, $p > .1$).

Since the same movies were repeatedly presented during the survey encoding, route encoding, and dot-control tasks, it is important to assess the effects of repetition on the BOLD response, i.e. repetition suppression during repeated presentations of the two experimental spatial encoding tasks (in contrast to the dot-control task).[1] To address this, we combined the beta estimates from the hippocampal ROI in the following manner: in each subject we collapsed the six dot-control task repetitions into pairs to provide three time points (Reps 1–2, Reps 3–4 and Reps 5–6). We then combined the beta estimates obtained during the repeated presentations of the spatial encoding tasks (survey and route) in the same manner. The end result was hippocampal beta estimates for each subject for multiple repetitions of the dot-control and the experimental spatial encoding tasks (see supplementary Figure S1a and S1b for an across subject average). There was no main effect of genotype or repetition ($p > .30$). There was a main effect of task, F(1, 114) = 12.44, $p < .001$. There was neither a Genotype × Repetition,

[1] We thank an anonymous reviewer for this suggestion.

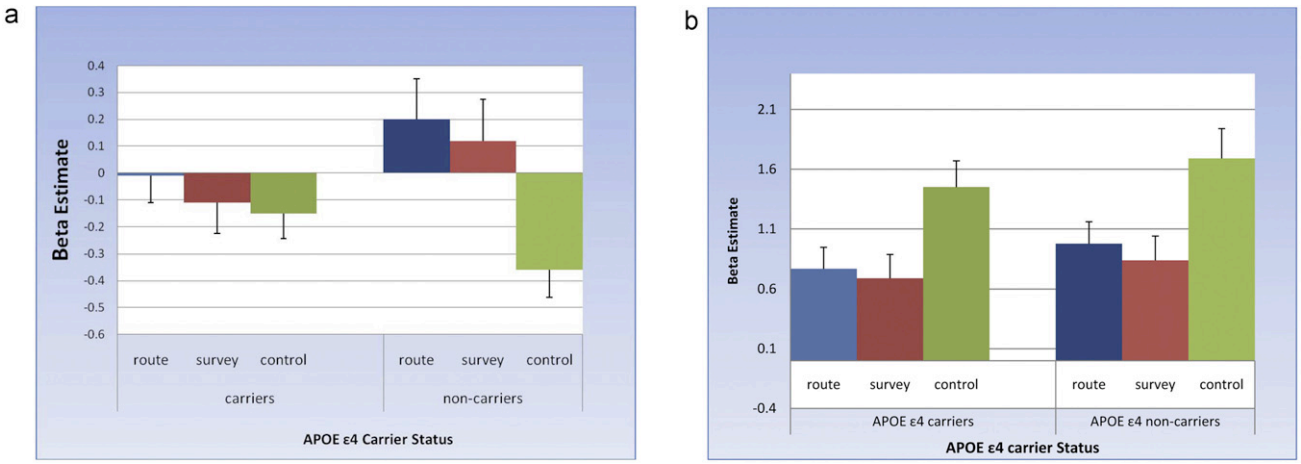

Fig. 2. (a) ROI analysis: Hippocampus estimated BOLD response: the mean estimated blood oxygen level dependent (BOLD) response in APOE $\varepsilon 4$ carriers and non-carriers during the survey encoding, route encoding, and dot-control tasks in bilateral hippocampus. Error bars designate standard error. (b) ROI analysis: estimated BOLD response: The mean estimated blood oxygen level dependent (BOLD) response in APOE $\varepsilon 4$ carriers and non-carriers during the survey encoding, route encoding, and dot-control tasks in the left lingual gyrus/calcarine fissure (BA 17/18). Error bars designate standard error.

Repetition × Task nor a Genotype × Repetition × Task interaction ($Fs < 1$). There was an overall Genotype × Task interaction, $F(1, 114) = 7.79$, $p < .01$, reflecting reduced encoding-related activation for the carriers compared to the non-carriers. Therefore, the APOE $\varepsilon 4$ effect on hippocampal activity seemed to be present from the earliest presentations and maintained throughout.

To confirm that the reduced encoding-related activation (relative to dot-control) in APOE $\varepsilon 4$ carriers did not reflect a global effect, we examined APOE $\varepsilon 4$-related activity in left lingual gyrus/calcarine fissure of the visual cortex (BA 17/18; Fig. 2b). In this *a priori* defined anatomical ROI, there was no main effect of genotype (F (1, 19) = .43, $p > .1$), nor an interaction between genotype and task (F (2, 38) = .09, $p > .1$). When the analysis was restricted to the two spatial learning conditions, there was no genotype × perspective interaction ($F(1, 38) = 0.01$, $p > .1$).

3.3.2. Activation differences in the whole brain

Our second hypothesis stated that APOE $\varepsilon 4$ carriers will show reduced activation, relative to non-carriers, in regions beyond the hippocampus proper – includes components of the DMN – –that are involved in effective encoding of route and survey perspectives compared to the dot-control task. The subsystems of the DMN include parts of the MTL, medial prefrontal cortex, posterior cingulate cortex, precuneus, and the medial, lateral and inferior parietal cortex (Pihlajamaki & Sperling, 2009). Therefore, in line with our hippocampal analysis above, we conducted whole brain (voxel-level) analyses, first examining task differences and then testing for interactions between task and APOE $\varepsilon 4$ status (with task as a within-subject factor and genotype as a between-subjects factor).

Task-related activation differences: First, we looked for regions displaying greater activity for the two encoding perspectives relative to the dot control task. These regions included bilateral middle temporal gyrus (BA 21), right posterior cingulate (BA 30/31), left superior parietal and precuneus (BA 7), left middle occipital gyrus (BA 19), right inferior frontal (BA 47), middle frontal (BA 9), superior frontal (BA 4), bilateral medial frontal gyrus (BA 11), and left postcentral gyrus (BA 5) in the uncorrected contrast ($p < .001$). The following regions survived correction at $p < .05$: left middle temporal gyrus (BA 21), right posterior cingulate (BA 30/31), left middle occipital gyrus (BA 19), right inferior frontal gyrus (BA 47), right middle frontal (BA 9), right superior frontal gyrus (BA 4), and bilateral medial frontal gyrus (BA 11). There was no significant difference between route and survey encoding at a statistical threshold of $p < .05$, corrected.

APOE $\varepsilon 4$-related activation differences: No regions in the brain demonstrated a significant interaction between genotype and spatial encoding perspective (route and survey). Similar to the hippocampal analysis described above, we then combined the data from the two perspectives and examined $\varepsilon 4$-related differences in BOLD response during spatial learning compared to the dot-control task. In the uncorrected contrast ($p < .001$), APOE $\varepsilon 4$ carriers demonstrated reduced encoding-related activation relative to the non-carriers in left cingulate cortex (BA 24), right cingulate cortex (BA 30), right superior frontal gyrus (BA 4), left middle frontal gyrus (BA 9), right supramarginal gyrus (BA 40), left insular cortex (BA 13), left anterior prefrontal cortex (BA 10), left inferior frontal gyrus (BA 47), right parahippocampal gyrus (BA 34), left hippocampus, and right superior temporal gyrus (BA 22). (See supplementary Table S1 for uncorrected results at $p < .001$). None of these individual regions survived correction at $p < .05$. There were no areas that showed greater activation during spatial encoding vs. dot-control in $\varepsilon 4$ carriers compared to non-carriers at either the uncorrected ($p < .001$) or corrected contrast, $p < .05$.

To explore how the two groups' BOLD activity varied during fixation and the dot-control task, we conducted a whole-brain analysis comparing BOLD activity during the dot-control task vs. fixation. When the dot-control task was directly compared to fixation, the mean BOLD response in $\varepsilon 4$ carriers was greater than that in non-carriers in right supramarginal gyrus, supplementary motor area, and right dorsal anterior cingulate cortex. Similar to results above, this activation also did not survive correction ($p < .05$). Relative to $\varepsilon 4$ carriers, non-carriers did not show a greater response during dot-control vs. fixation in any brain region (corrected at $p < .05$).

4. Discussion

The present study investigated hippocampal activation in cognitively normal older adults as they engaged in a perspective-dependent learning task. Consistent with the primary hypothesis, APOE $\varepsilon 4$ carriers showed less encoding-related activation in the hippocampus proper compared to non-carriers. That is, when the encoding of the two spatial perspectives was contrasted against the dot-control task the encoding-related activation was significantly lower in carriers than non-carriers. Lower activation, however, was not global as encoding-related activation was not different between APOE $\varepsilon 4$ carriers and non-carriers in the bilateral middle occipital region. In addition, regions implicated in effective encoding across the whole brain (inclusive of neural components of the

DMN) showed APOE ε4-related differences during the perspective-dependent learning tasks as predicted. These neural differences were observed even though memory performance was comparable across carriers and non-carriers.

4.1. APOE ε4 and the default mode network

The DMN is altered in cognitively normal older APOE ε4 carriers similar to MCI and AD patients (Filippini et al., 2009; Fleisher et al., 2009; Persson et al., 2008; Pihlajamaki & Sperling, 2009; Pihlajamaki et al., 2010; Rombouts, Barkhof, Goekoop, Stam, & Scheltens, 2005). Lustig et al. (2003) reported that while responses in medial parietal and posterior cingulate regions went from activation during a semantic judgment task to deactivation during fixation in young participants, these regions were consistently activated in older adults with AD. Pihlajamaki and Sperling (2009) provided evidence for the disruption of DMN along the continuum from normal aging to APOE ε4 carriers to MCI and then AD. Recently, Fleisher et al. (2009) reported no encoding-related activity differences in ε4 carriers compared to non-carriers during a novel face-name pair task. Encoding-associated deactivations in the medial and right lateral parietal cortex were greater in non-carriers, similar to findings in AD studies. Fleisher et al. (2009) also did a resting-state DMN analysis that revealed nine regions in lateral prefrontal, orbital frontal, temporal and parietal cortices that were different between ε4 carriers and non-carriers. The present study revealed APOE ε4 encoding-related differences (ε4 carriers < non-carriers, $p < .001$, uncorrected) in orbital frontal and temporal lobe areas. Caution must be taken as these areas did not survive a more conservative threshold. These areas, however, are argued to be components of the DMN, with recent data revealing that resting state activity in this region differs between carriers and non-carriers (Fleisher et al., 2009). In addition, a previous study also reported that the pattern of altered task-induced deactivations in APOE ε4 carriers is similar to the DMN (Persson et al., 2008).

It is possible that the ε4-related difference in the present study is driven by preclinical atrophy in the hippocampus and surrounding areas. The underlying structural atrophy of these regions (hippocampal, surrounding MTL and orbital frontal cortex) may be the reason for the alteration in the DMN of ε4 carriers, MCI and AD as well as the reduction of encoding activity in APOE ε4 carriers compared to non-carriers in our study. Previous studies have shown that although elderly ε4 carriers show some atrophy in the MTL, there is no global brain atrophy (den Heijer et al., 2002; Geroldi et al., 1999; Soininen et al., 1995). Fig. 2b provides evidence for this as it shows patterns of activity in the left lingual gyrus/calcarine fissure that differ from what is observed in the hippocampus, as there was neither a main effect of Genotype nor a Genotype x Task interaction.

4.2. Methodological considerations

A recent comprehensive review of studies investigating BOLD responses in APOE ε4 carriers during encoding reported that the data pertaining to the direction and location of the effect of APOE genotype on the BOLD response are inconsistent (Trachtenberg et al., in press; Wierenga & Bondi, 2007). Some of the reasons for these inconsistencies could be age differences in the participant population and methods for fMRI data analysis, including the use of high- versus low-level contrasts.

The present study methodologically differed from a recent, related study that reported reduced activation during both route and survey encoding in the MTL of older adult ε4 carriers (Borghesani et al., 2008). First, the participants in Borghesani et al. were, on average, older than our participants (mean ages of 72.4 vs. 63.8 yrs, respectively). Because the influences of APOE genotype on brain function may change with age (Alexander et al., 2007; Small et al., 2004), across-study differences may partially reflect age × genotype interactions. Future research is needed to determine whether and how the effects of genotype on spatial encoding processes change across the life span. Second, with regards to task-related differences, Borghesani et al. (2008) used a single environment for the two perspectives, such that participants may have used the two different perspectives to gather complementary information about the environment; that is, the learning effects during survey and route encoding were not independent. It is plausible to hypothesize that the processes engaged during survey or during route encoding will vary depending on whether an environment has or has not been previously encountered from the other perspective. Finally, the present study used a novel dot-control task as a low-level contrast (in addition to fixation), along with the high-level contrast of the two perspectives. Our data indicate that the dot-control task does not activate the hippocampus in either gene group relative to the fixation baseline (see the negative BOLD response during dot-control; Fig. 2a), suggesting that the processing demands for the dot-control task are less dependent on the hippocampus relative to the processes engaged during both fixation and during the two spatial encoding conditions. As such, the dot-control task may serve as a more effective baseline for detecting group differences in MTL-dependent spatial encoding processes, further underscoring the importance of the low-level control task when targeting complex encoding computations (Gusnard & Raichle, 2001; Stark & Squire, 2001)—specifically in cognitively normal young-old populations. Indeed, we observed an ε4-related difference in encoding activation when compared to the dot-control task, but not when compared to the fixation baseline in our older participants. Collectively, while our data provide clear evidence of a reduction in encoding-related neural responses in ε4-carriers, the methodological, including participants' age differences, between our experiment and that of Borghesani et al. (2008) complicate direct comparisons.

4.3. Task-related activation differences

Although the route vs. survey contrast did not reveal significant differences in functional activation in our study, the relative activity illustrated in Fig. 2a shows that the difference between the route and survey perspectives is in the direction observed in previous studies (route > survey; Shelton & Gabrieli, 2002). The present study employed a stringent threshold at which no areas were differentially activated when route perspective was directly compared with survey perspective, regardless of genotype. When a less stringent threshold was adopted ($p < .01$, uncorrected), left insular cortex (BA 13), left middle temporal gyrus (BA 39), left superior frontal gyrus (BA 4), and left middle occipital gyrus (BA 19) were more active in route compared to survey perspective. Left superior frontal gyrus (BA 4), precentral gyrus (BA 3), and right middle frontal gyrus (BA 9) were more active in survey than route perspective.

There are a number of limitations to this study. First, the relatively small number of participants likely resulted in modest power to detect within and between group differences, and also contributed to an under representation of women. Note however, that a sample size smaller than the present study (combined $n = 14$ compared to combined $n = 21$) reported significant APOE ε4 related differences in the MTL earlier (Borghesani et al., 2008). Second, our fixation blocks lasted only 10 s (total 18 fixation blocks), whereas the dot-control blocks were 20 s long (total six blocks). While it is clear that the dot-control task resulted in reduced MTL activity relative to fixation, nevertheless the briefer durations of the fixation blocks may have resulted in there being partially colored by hemodynamic carryover, thus leading the fixation blocks to have less power as a baseline comparison to the perspective-dependent

learning tasks. Third, while we note the relationship between the present effects and neural components of the putative DMN, we did not directly identify this network due to the limited amount of fixation data available. Future studies that use resting-state functional connectivity (fcMRI) measures to identify the DMN are necessary to more firmly establish the effects of ε4-carrier status on activation in this network. Finally, the present study revealed matched memory performance in the carrier and non-carrier groups, and thus does not document a behavioral correlate of the observed functional differences in encoding-related neural responses. A recent study used the subsequent memory paradigm (Brewer, Zhao, Desmond, Glover, & Gabrieli, 1998; Paller & Wagner, 2002; Wagner et al., 1998) to identify differences in event-related BOLD activity between subsequently forgotten and remembered items and observed that neural processing differences between ε4 carriers and non-carriers were related to their ability to encode items into long term memory (Dennis et al., 2009). Future studies of the effects of APOE status on spatial encoding processes should be designed to permit a linking of subsequent memory behavior to moment-to-moment measures of the BOLD response.

4.4. Conclusion

This study provides an additional viewpoint on APOE ε4-related activation differences in the hippocampus proper during encoding of a previously established perspective-dependent learning paradigm. The current study used the dot-control task to effectively target APOE ε4-related activity differences during the perspective-dependent learning tasks. Based on previous studies where DMN activity was reported to be disrupted in APOE ε4 carriers, the present study further highlights the role of low-level control tasks to tease apart the influence of genetic susceptibilities on brain activity during learning. This approach, when coupled with resting-state fcMRI analyses, may increase the chances of capturing clinically significant ε4-related differences in middle aged to older population, and may yield better models to determine the influences of various moderators, like age, education, and general intelligence, on the neural substrates of episodic encoding.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuropsychologia.2011.04.022.

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