ORIGINAL ARTICLE

Interactive Effect of Apolipoprotein E Genotype and Age on Hippocampal Activation During Memory Processing in Healthy Adults

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Context: Although the apolipoprotein E (*APOE*) ε4 allele is a major genetic risk factor for late-onset Alzheimer disease, its effect on hippocampal function during episodic memory is controversial because studies have yielded mixed results. The age of the studied cohorts may contribute to this apparent inconsistency: activation for ε4 carriers tends to be increased in studies of older adults but decreased in some studies of younger adults. Consistent with differential age effects, research in transgenic mice suggests that the ε4 allele may particularly affect the aging process.

Objective: To define the interactions of age and this allelic variation on brain activation during episodic memory across adult life in healthy individuals.

Design: Functional magnetic resonance imaging (fMRI) using an episodic memory paradigm to test for differences in neuroactivation across *APOE* genotypes and age groups.

Setting: A federal research institute.

Participants: Healthy white volunteers (*APOE* ε3 homozygotes and ε2 and ε4 heterozygotes) completed the fMRI task (133 volunteers aged 19-77 years).

Main Outcome Measure: Memory-related regional blood oxygenation level–dependent (BOLD) activation.

Results: Genotype affected the pattern of change in hippocampal BOLD activation across the adult lifespan: older age was associated with decreased activation in ε2 carriers and, to a lesser extent, in ε3 homozygotes, but this pattern was not observed in ε4 carriers. Among young participants, ε4 carriers had less hippocampal activation compared with ε3 homozygotes despite similar task performance.

Conclusions: The findings support the hypothesis that aging and *APOE* allele status have interacting effects on the neural substrate of episodic memory and lend clarification to disparities in the literature. The stepwise decrease in activation with age found among genotype groups resembles the order of susceptibility to Alzheimer disease, suggesting a compensatory neurobiological mechanism in older asymptomatic ε4 carriers.

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APOLIPOPROTEIN E (APOE), a transporter of cholesterol and lipids, plays a critical role in the brain, where it is involved in lipid homeostasis and neuronal repair.[1] The 3 major alleles of *APOE* (ε2, ε3, and ε4) produce 3 major isoforms (APOE2, APOE3, and APOE4). The *APOE* ε4 allele is a major genetic risk factor for late-onset Alzheimer disease (AD), significantly increasing risk and reducing age at onset,[2] likely because APOE4 is less effective in protecting neurons from age-associated oxidative damage, in the proteolytic degradation of amyloid β, and in promoting cholesterol transport.[1]

To explore the potential mechanisms of how *APOE* ε4 predisposes to AD, characterized by early memory loss, the effect of this allele on brain activation by memory tasks has been studied[3-13] in healthy individuals using functional magnetic resonance imaging (fMRI). The results have been divergent, with findings of both greater and lesser medial temporal lobe (MTL) activation. A review of the literature suggests that, at least in part, this divergence could be related to differing ages of the studied cohorts, which have tended to contain either young or older participants, but not both. Several of the larger studies[8,9,11-13] of young *APOE* ε4 carriers have reported reduced MTL activation, while 2 recent studies[12,13] with smaller samples of young carriers report greater activation.[8,9,11-13] Larger studies[3,4,6,7,10] of older carriers have found increased activation. However, there has been no sufficiently powered investigation of the effect of this allelic variation across the adult

lifespan: 2 studies[10,14] reported contradictory, trend-level MTL findings.

In contrast to the *APOE* ε4 allele, the *APOE* ε2 allele is associated with decreased risk of AD.[15,16] This genetic variant has been studied in cell and mouse models, but there has been little investigation of its effects on human brain function during cognition,[9,17] likely the result of its low prevalence.

In the present study, we examined the effect of the *APOE* ε4 allele on brain activation during performance of an fMRI episodic memory task in a large sample of healthy participants (N = 133) across the adult age span. We also included *APOE* ε2 carriers to gain insight into the neural underpinnings of the reported protective effects of this allele. We hypothesized that, particularly in the MTL, where potential excitotoxicity and neuronal repair processes are most prominent,[18,19] *APOE* genotype would differentially affect changes in memory-related neural recruitment across the adult age span and that, across genotype groups, there would be a stepwise function describing the relationship between age and activation that reflects relative risk for AD conferred by the 3 *APOE* alleles.

Additionally, given the small number and mixed findings of published studies in young individuals,[8,9,11-13] we took advantage of our relatively large cohort to address this open issue by testing for an effect of the *APOE* ε4 allele on MTL activation in a subset of participants aged 40 years or younger. A similar analysis was performed in participants older than 50.

Finally, memory performance across *APOE* genotypes has been extensively studied in older individuals, but not in younger ones. A recent meta-analysis[20] listed 76 studies, but the mean age was younger than 40 years in only 2 of the studies and the results were contradictory. To further elucidate this issue and to characterize the population from which our imaging cohort was drawn, we studied memory performance outside the scanner in a larger young sample, including most of the participants in our fMRI study.

METHODS

PARTICIPANTS

One-hundred thirty-three healthy, right-handed white volunteers, aged 19 to 77 years, participated in an fMRI episodic memory task. To test memory performance outside the scanner in young adults, we studied 197 healthy volunteers from the same study cohort, aged 18 to 40 years. Of them, 87 also participated in our fMRI activation study.

All participants provided written informed consent for the study, which had been approved by the institutional review board of the National Institute of Mental Health. Participants were administered the Structured Clinical Interview for *DSM-IV-TR* Disorders[21] to rule out psychiatric illness. We excluded people taking psychotropic medications or any other drugs that might interfere with brain activity/blood flow, as well as individuals having a history of head injury with loss of consciousness. To rule out medical illness, participants were assessed with physical and neurologic examination, laboratory studies, and structural MRIs. Individuals who had white matter hyperintensities qualifying for a grade greater than 0 in the Scheltens scale were also excluded.[22] Only white participants were included to minimize population stratification and because of documented racial differences in the effect of the *APOE* ε4 allele on risk for AD and outcome following injury.[23,24] Only right-handed participants (Edinburgh score ≥50%) were included to reduce variability due to hemispheric differences in information processing.[25] We included homozygous ε3 carriers and heterozygous ε2 (2/3) and ε4 (3/4) carriers, which, when combined, represent more than 90% of the white population (55%, 12%, and 25%, respectively).[26] The *APOE* genotypes 2/2, 2/4, and 4/4 were excluded because they are infrequent, pose a different risk for AD, and are likely to differ in neurobiological features.[23] We included all eligible participants in the study, resulting in an allele frequency distribution that is similar to that in the white population at large. Demographic variables and measures of recall were assessed with an analysis of variance, and sex differences were tested with a χ^2 test.

GENOTYPING

The *APOE* genotyping was done by 5′-exonuclease allele–specific fluorescent detection method (TaqMan, Applied Biosystems).[27] Custom probes were designed (Applied Biosystems) for *APOE* 112 and *APOE* 158 polymorphisms and ε2, ε3, and ε4 haplotypes were derived from these 2 genotypes.

NEUROPSYCHOLOGICAL ASSESSMENT OF RECALL

A subset of participants completed neuropsychological measures outside of the MRI scanner. The measures included the Wechsler Memory Scale Logical Memory I and II to assess immediate and delayed recall.[28]

fMRI DATA ACQUISITION

Functional MRI was obtained using a 3-T scanner (Signa; General Electric). Blood oxygenation level–dependent (BOLD) images were acquired with a gradient echo-planar imaging sequence and covered 24 axial sections (4-mm thickness, 1-mm gap), using the following parameters: repetition time, 2000 milliseconds; echo time, 28 milliseconds; field of view, 24 cm; and matrix, 64 × 64 matrix. These parameters were selected to optimize the quality of the BOLD signal while allowing for a sufficient number of sections to acquire whole-brain data. Acquisition parameters were described in greater detail in a previous publication.[29]

fMRI EPISODIC MEMORY TASK

Each participant underwent BOLD fMRI during the encoding and retrieval of neutral scenes selected from the International Affective Picture System.[30] The scenes were presented in a blocked fashion, with 4 encoding blocks interleaved with 5 fixation blocks, followed after 2 minutes by 4 retrieval blocks, which were also interleaved with fixation blocks. Each block was 20 seconds long. During fixation blocks, which were treated as baseline in the fMRI analyses, participants were asked to attend to a fixation cross presented in the center of the screen. During each encoding block, 6 novel scenes were presented serially, each for 3 seconds, and participants were instructed to determine whether each picture depicted an indoor or an outdoor scene, with 3 of each type presented. During retrieval blocks, participants were instructed to determine whether the scene presented had been shown during the encoding blocks; half the scenes had been presented and half were novel. Participants were instructed to use their dominant hand to press the right button for scenes already seen during encoding (*old*) or press

the left button for scenes not seen during the encoding session (*new*). Following previous use,[29] we referred to this second portion of the task as *retrieval*, with the proviso that the BOLD effects recorded then may not entirely reflect retrieval but could be associated with the presentation of novel images and the discrimination between old and new images. Behavioral accuracy and reaction times were recorded.

fMRI DATA ANALYSIS

Image Processing

Using SPM5 (Wellcome Trust Centre for Neuroimaging), images were realigned to the first saved volume in the time series and to an average volume and were spatially normalized to a standard stereotactic space (Montreal Neurological Institute [MNI]) using a 12-parameter affine transformation followed by a nonlinear warp using cosine basis functions, with the smallest wavelength being 25 mm. Regularization was applied when estimating the optimal transformation to penalize excessive warping, and interpolation was fourth-order β-spline.[31] Images were then spatially smoothed with a gaussian filter of 8 mm^3 full width at half maximum. Quality control included visual inspection for image artifacts, estimating indices for ghost artifacts, assessing signal to noise ratio across the time series, measuring signal variance across individual sessions, and restricting head motion.[32] We used the general linear model to assess fMRI responses, with a canonical hemodynamic response function convolved to a boxcar function for the length of the block. Data were normalized to the global signal across the whole brain and temporally filtered to remove low-frequency signals. Regressors were modeled for conditions of interest as well as for 6 head-motion regressors of no interest.[32]

Second-level random-effects analyses were carried out to (1) assess the effect of *APOE* genotype on the relationship between age and functional activation during encoding and retrieval in participants across the age range 19 to 77 years and (2) determine the effect of genotype on activation within 2 subgroups, a younger one (age, 19-40 years) and an older one (age, 51-77 years). For these analyses, a mask generated from a contrast of the main effect of task across all participants (using a threshold of $P<.05$, uncorrected) was used so that assessment was restricted to task-relevant regions. In addition to analyses in task-relevant regions throughout the brain, we conducted analyses restricted to volumes of interest of the hippocampus and parahippocampus, delineated using the PickAtlas toolbox.[33,34] Herein, we report only findings significant at a voxel-based threshold of $P<.05$, familywise error (FWE) corrected. Coordinates are reported in MNI space.

Effect of Genotype on the Relationship Between Age and Activation

Two approaches were taken to assess the relationship between genotype and age-related changes in activation among our larger group of participants aged 19 to 77 years: one data driven and one hypothesis driven. First, in the data-driven analysis, we performed a voxelwise regression analysis across the entire sample (N=133), with age as the single regressor, to identify regions showing a significant age-related change in activation, regardless of genotype group. To gain insight into how this age-related effect broke down by genotype group, we extracted the activity values for each participant at the left and right hippocampal maxima in the age-activation correlation for the entire group and determined the slope of the relationship between age and activation for each genotype group in post hoc examinations using the Spearman correlation coefficient (Statistica 6.0; StatSoft).

Second, the hypothesis-driven analysis was designed to test for a stepwise function in the relationship between age and activation that reflects relative risk conferred by the 3 *APOE* genotypes. For this purpose, we performed a voxelwise analysis of covariance with 3 age covariates, 1 for each genotype group, and contrasted the effect of age on activation for ε4 greater than the effect of age on activation for ε3 inclusively masked with the contrast age on activation for ε3 greater than age on activation for ε2 (effectively, ε4>ε3>ε2) as well as the inverse contrast, ε2>ε3 masked with ε3>ε4 (effectively, ε2>ε3>ε4), with a combined statistical threshold of $P<.001$. To confirm and better visualize the stepwise effect, we constructed a plot of the underlying correlation between age and activation for each genotype group in a 6-mm sphere centered on the maximum voxel derived from the primary ε4>ε3>ε2 activation analysis and determined the corresponding Spearman correlation coefficients.

Finally, we performed simple comparisons between the age-activation correlation maps of each pair of genotype groups using voxelwise unpaired, 2-tailed *t* tests. We used the same statistical threshold and again restricted reported findings to task-relevant regions.

Effect of Genotype on Activation in Younger and Older Subgroups

We examined the effect of genotype on activation within 2 age subgroups: 19 to 40 years (n=91) and 50 to 77 years (n=26) (**Table 1**). In the younger sample we performed a voxelwise analysis of variance to determine activation across the 3 genotypes. In the older group, we performed a voxelwise *t* test comparing BOLD activation between ε3 homozygotes and ε4 carriers; the small number of older ε2 carriers prevented us from including this genotype group.

RESULTS

DEMOGRAPHICS, PERFORMANCE, AND OVERALL TASK ACTIVATION PATTERN

Genotype groups did not differ significantly in age, educational level, or IQ (Table 1). There were no significant between-group differences in fMRI task performance or in the relationship between age and performance for encoding ($P=.96$) or retrieval ($P=.44$). Among young participants, there was a trend for faster reaction time (time to button press) during fMRI among ε3 homozygotes ($P=.051$) during encoding, although performance scores between genotype groups were equivalent. In the behavioral study of young participants performed outside the scanner, *APOE* ε2 and ε4 carriers performed better than *APOE* ε3 homozygotes on Logical Memory I ($P=.02$) and Logical Memory II ($P=.04$), tests of immediate and delayed recall of the Wechsler Memory Scale–Revised (**Table 2**).

Consistent with previous studies,[29,32] in all participant groups during both encoding and retrieval, our fMRI episodic memory task elicited robust activation of the hippocampal formation; prefrontal, parietal, occipital, and inferotemporal cortices; cerebellum; putamen; and thalamus (eFigure 1; available at http://www.archgenpsychiatry.com).

Table 1. Demographic and Performance Data for Participants of the fMRI Episodic Memory Task[a]

Demographics and Performance	*APOE* Genotype ε2/ε3[b] (n = 17)	ε3/ε3 (n = 81)	ε3/ε4 (n = 35)	*P* Value[c]
All Participants, Aged 19-77 y (N = 133)				
Sex, M/F	5/12	36/45	15/20	.52
Age, y	37 (15.6)	35 (14.5)	38 (16.7)	.69
WAIS score	112 (12.5)	110 (8.2)	108 (12)	.45
Educational level, y	17 (1.9)	17 (2.5)	17 (1.9)	.74
Correct ENC, %	95 (3.5)	94 (6.2)	94 (5.9)	.59
ENC RT, ms	1286 (107.2)	1237 (157.7)	1259 (132.8)	.43
Correct RET, %	88 (7.2)	85 (8.8)	83 (14.4)	.35
RET RT, ms	1467 (176)	1423 (154.3)	1432 (130.6)	.59
Young Participants, Aged 19-40 y (n = 91)				
Sex, M/F	2/9	24/33	9/14	.33
Age, y	27 (3.4)	27 (5.3)	27 (4.9)	.98
WAIS score	108 (10.1)	108 (7.2)	104 (11.9)	.14
Educational level, y	17 (1.4)	17 (2.4)	17 (1.5)	.86
Correct ENC, %	95 (3.7)	94 (6.8)	94 (6.7)	.76
ENC RT, ms	1291 (107.7)	1211 (136.3)	1281 (138.3)	.051
Correct RET, %	91 (6)	85 (8.6)	83 (17)	.19
RET RT, ms	1463 (188)	1399 (135.9)	1433 (125.8)	.32
Older Participants, Aged 51-77 y (n = 26)				
Sex, M/F	. . .	9/8	4/5	.50
Age, y	. . .	59 (5.3)	63 (6.6)	.17
WAIS score	. . .	114 (7.9)	117 (9.6)	.49
Education, y	. . .	17 (2.5)	16 (2.4)	.31
Correct ENC, %	. . .	93 (5.1)	95 (4.1)	.35
ENC RT, ms	. . .	1285 (170.4)	1197 (116.4)	.21
Correct RET, %	. . .	84 (8.8)	81 (6.3)	.32
RET RT, ms	. . .	1482 (170.6)	1421 (162.5)	.39

Abbreviations: *APOE*, apolipoprotein E; ENC, encoding; fMRI, functional magnetic resonance imaging; RET, retrieval; RT, reaction time; WAIS, Wechsler Adult Intelligence Scale.

[a] Data are given as mean (SD) unless indicated otherwise.

[b] The small number of older ε2 carriers prevented us from including that genotype group in the analysis of the older participants.

[c] Demographic measures were analyzed in an analysis of variance and sex differences using a χ^2 statistic.

EFFECT OF GENOTYPE ON THE RELATIONSHIP BETWEEN AGE AND ACTIVATION

In our data-driven regression analysis of the effect of age on BOLD activation during encoding for all 133 fMRI study participants (age, 19-77 years), we found a significant decline in activation with increasing age in the posterior hippocampus bilaterally ($P<.0001$, FWE-corrected volumes of interest and whole brain, **Figure 1**). When activation data from the left hippocampal maximum voxel (MNI *x*, *y*, and *z*=–22, –22, and –8) were extracted and analyzed by genotype group, we found that the greatest decline in activation with age occurred among ε2 carriers (Spearman ρ, $R=-0.8$; $P<.001$), followed by ε3 homozygotes ($R=-0.41$; $P<.0001$), and finally ε4 carriers, whose hippocampal activation showed no significant relationship with age ($R=-0.23$; $P=.19$). This pattern was also observed for the right hippocampal voxel maximum (MNI *x*, *y*, *z*=22, –30, –4: ε2 $R=-0.79$, $P<.0001$; ε3 $R=-0.58$, $P<.0001$; and ε4 $R=0.05$, $P=.79$) (Figure 1). Additional areas in which there was a decline in activation with increasing age for the entire group included the fusiform gyrus bilaterally and the lateral occipital gyrus, BA19 (eTable). There were no regions in which a significant increase in activation with age was observed at the thresholds reported.

Table 2. Results of Memory Testing Outside the Scanner for 197 Younger Participants[a]

Demographics and Performance	*APOE* Genotype ε2	ε3	ε4	*P* Value[b]
Sex, M/F	10/17	56/66	16/32	
WMS-R Logical Memory I	31 (5.5)	28 (5.6)	30 (6.5)	.015
WMS-R Logical Memory II	27 (5.9)	24 (6.2)	26 (7.4)	.037

Abbreviations: *APOE*, apolipoprotein E; WMS-R, Wechsler Memory Scale–Revised.

[a] Young (18-40 years) *APOE* ε2 and ε4 carriers performed better than young ε3 homozygotes on logical Memory I and Logical Memory II tests of immediate and delayed recall of the WMS-R. Data are given as mean (SD) unless indicated otherwise.

[b] *P* value was calculated with analysis of variance.

During retrieval, we again observed a significant decline in activation with increasing age in the posterior hippocampus bilaterally ($P<.001$, FWE corrected, volume of interest and whole brain) (eFigure 2), with a greater decrease among ε2 carriers and ε3 homozygotes in the right (MNI *x*, *y*, and *z*=19, –34, and 0: $R=-0.38$,

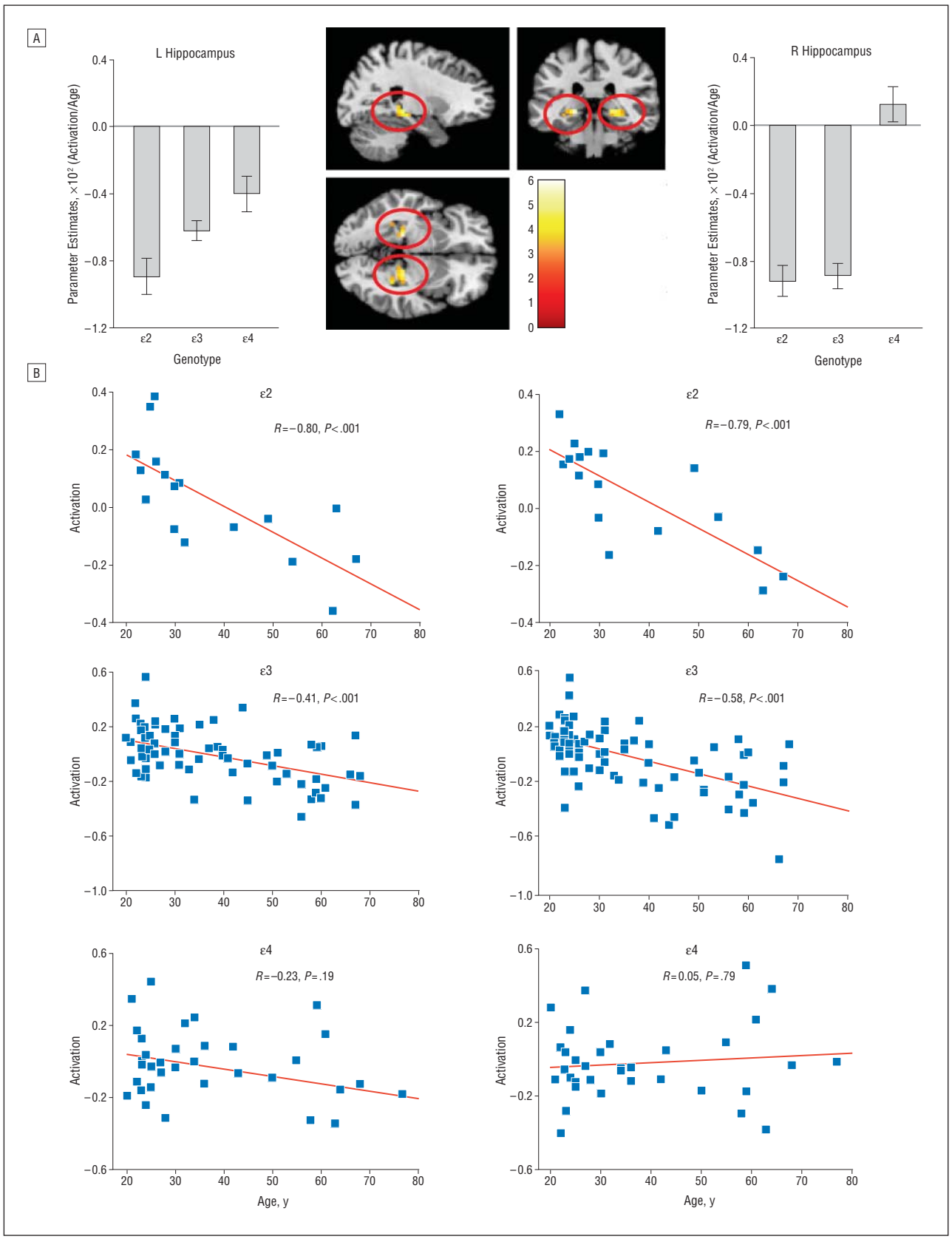

Figure 1. Decreased blood oxygenation level–dependent activation with increasing age. On 3 orthogonal magnetic resonance imaging templates, areas in yellow-red have decreased activation with increasing age during memory encoding ($P<.05$ familywise error corrected) (N=133). At the maximal voxel for the right (x, y, and z=22, −30, and −4) and left (−22, −22, and −8) hippocampi, parameter estimates (A) and scatterplots (B) are shown for the correlation of activation with age for each genotype. Limit lines indicate SD. L indicates left; R, right.

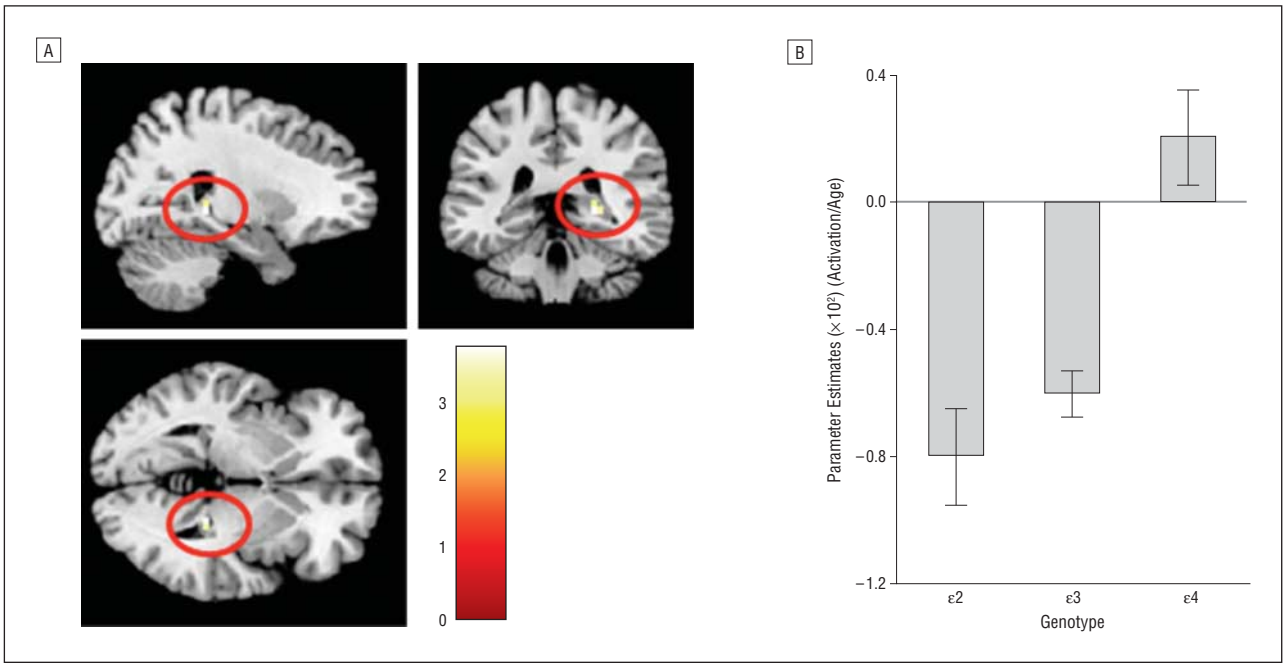

Figure 2. Decreased blood oxygenation level–dependent activation with age as a stepwise function of genotype. A, On 3 orthogonal magnetic resonance imaging templates, the area of the right hippocampus displayed in yellow had decreased activation with increasing age during memory encoding as a stepwise function of genotype (ε4 > ε3 > ε2, *P*=.02, familywise error corrected) (N=133). B, Parameter estimates for the maximal voxel (x, y, and z=22, –34, and 0). Limit lines indicate SD.

P=.13; and *R*=–0.54, *P*<.001, respectively) and left (MNI x, y, and z=–19, –30, and –4: *R*=–0.82, *P*<.001; and *R*=–0.41, *P*<.001) hippocampi, and no such relationship among ε4 carriers (*R*=–0.26, *P*=.13; and *R*=–0.21, *P*=.22, respectively). Additional (nonhypothesized) areas showing a decline in activation with increasing age for the entire group included the left fusiform gyrus and the supplementary motor area bilaterally (eTable). Again, there were no regions in which a significant increase in activation with age was observed.

Our hypothesis-driven analysis, designed to search for ε4>ε3>ε2 effects, defined a region during encoding in the right posterior hippocampus where there was a significant stepwise change across genotype groups in the relationship of activation with age (MNI x, y, and z=22, –34, and 0: *P*=.02, FWE corrected, **Figure 2**). A post hoc analysis of age and activation using average activity values extracted from this region revealed a significant decline in activation with age among ε2 carriers and ε3 homozygotes, with the greatest decline among ε2 carriers (*R*=–0.68, *P*=.003; and *R*=–0.47, *P*<.001, respectively), but no such relationship between age and activation in ε4 carriers (*R*=0.06, *P*=.74). The findings mirror those from our analysis of the effect of age alone. There were no significant effects during retrieval and no regions in which the opposite relationship, ε2>ε3>ε4, was observed for either encoding or retrieval.

Our voxelwise comparison between age-activation maps of each pair of genotype groups for participants aged 19 to 77 years revealed that during encoding, there was a significant difference between ε4 carriers and ε3 homozygotes in the relationship between age and activation in the right posterior hippocampus (MNI x, y, and z=26, –34, and –8: *P*<.05, FWE corrected, volume of interest [*P*=.002], and whole brain [*P*=.04]). Similarly, when ε4 carriers were compared with ε2 carriers, a differential effect of age was observed, again in the right posterior hippocampus (x, y, and z=22, –30, and 0: *P*=.03, FWE corrected). In this pairwise, between-group analysis, no significant between-genotype differences in the relationship between age and activation were observed during retrieval at the statistical thresholds reported.

EFFECT OF GENOTYPE ON ACTIVATION IN THE YOUNGER SUBGROUP

During encoding, ε4 carriers demonstrated less activation than ε3 homozygotes in the posterior portion of the hippocampal and parahippocampal gyri bilaterally (x, y, and z=–30, –34, and –11, *P*=.03; and 26, –34, and –8, *P*=.009, respectively, FWE corrected) (**Figure 3** and eTable). We observed less activation of the left pericalcarine cortex, Brodmann area (BA) 18, among ε3 homozygotes when compared with ε2 heterozygotes (eTable). During retrieval, ε4 carriers showed a trend for decreased activation of the right hippocampus/parahippocampus (eTable) compared with ε3 homozygotes.

GENOTYPE EFFECTS IN THE OLDER SUBSET

We observed a trend toward greater activation among older ε4 carriers compared with older ε3 homozygotes in the right medial temporal lobe during both encoding (centered in the subiculum; x, y, and z=19, –22, and –11; *P*<.009 uncorrected) and retrieval (centered in CA1; x, y, and z=41, –19; and –15; *P*<.008 uncorrected).

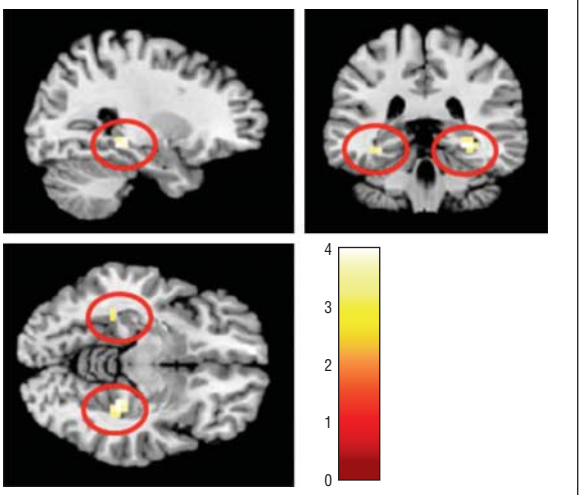

Figure 3. Decreased blood oxygenation level–dependent activation in young ε4 carriers compared with ε3 homozygotes. On 3 orthogonal magnetic resonance imaging templates, displayed in yellow are the areas where ε4 carriers aged 40 years or younger (n=91) demonstrated less activation ($P<.05$, familywise error corrected) of the hippocampi (right, x, y, and z=26, –34, and –8; left, –30, –34, and –11) during memory encoding compared with young ε3 homozygotes.

COMMENT

Our data-driven and hypothesis-driven analyses converge on the finding that *APOE* genotype affects medial temporal lobe activation during an fMRI episodic memory task but does so in a predictable, age-dependent manner. For ε4 carriers, there was no significant correlation between activation and age during encoding and retrieval, whereas hippocampal recruitment significantly decreased with increasing age among ε3 homozygotes and did so even more steeply among ε2 carriers. This age × genotype effect, which, in its stepwise fashion (Spearman ρ for ε4 > ε3 > ε2), resembles the order of susceptibility to AD among genotypes, has not been previously described.

These findings are consistent with those of earlier studies suggesting that, in older individuals, greater hippocampal activation occurs among ε4 carriers; however, we found that this relationship is driven by a significant decline in activation with age among ε3 homozygotes and ε2 carriers. Because these 2 genotypes are not only more resistant to AD[2] but also tend to have better memory function in older age,[4,23] it is of interest to consider the implications of our finding of less MTL activation with age. Because these 2 genotypes constituted the larger portion (73.7%) of our sample, we can evaluate similar activation studies performed in healthy individuals from a similar background, even if genotyping was not carried out. In agreement with our finding, studies comparing memory encoding in younger and older participants almost uniformly report less MTL activation in older persons, both with positron emission tomography[35-37] and fMRI.[38-42] For retrieval, age-related changes in activation have been less consistently observed. The neurobiological mechanism for decreased MTL activation with increasing age remains to be clarified. A pattern of "posterior-anterior shift in aging" has been described: across a variety of different cognitive tasks, less activation of posterior structures, including the temporal lobe, has been observed in older healthy individuals, who instead have greater frontal activation.[43-45]

Our finding of a genotype effect on age-related MTL activation across adulthood had been explored by 2 studies.[10,14] The larger of these investigations detected an area of the right hippocampus in which the authors reported a trend for an age × genotype interaction.[10] There, the tendency was for non–ε4 carriers to have decreased activation with increasing age, whereas ε4 carriers without a family history of AD tended to have a mild increase with age, both tendencies in the same direction as our findings. In the other study, an age × genotype interaction did not elicit voxel-based findings in the MTL, possibly because of the small sample size.[14]

The interpretation of age-related changes in BOLD activation is complex, since many factors may influence the amplitude and extent of the BOLD signal with aging.[46-48] For instance, age-related hippocampal atrophy[49] could cause the inclusion of a greater proportion of cerebrospinal fluid in the volume of study and therefore result in reduced BOLD signal in older individuals.[50] However, because older ε4 carriers are reported to have more MTL atrophy than are ε3 homozygotes,[51,52] this mechanism would be expected to yield a greater decline in MTL activation with aging in the ε4 carriers, rather than less decline as we observed. Therefore, the most plausible explanations for our findings are that, with advancing age, ε4 carriers do not demonstrate adaptive changes in the brain or they require a greater recruitment of MTL neuronal resources to accomplish equivalent memory encoding. Greater activation as a compensatory mechanism has been documented in older individuals with mild cognitive impairment, often a precursor to AD: greater posterior hippocampal activation during scene encoding predicted subsequent cognitive decline.[53,54] Scene encoding, which was used in this experiment, preferentially activates the posterior, rather than the anterior, portion of the hippocampal formation. The greater hippocampal activation that we observed in older ε4 carriers, when contrasted with ε2 and ε3 carriers, is compatible with the finding in this genotype of upregulated mitochondrial oxidative phosphorylation/energy metabolism in a gene transcription study[55] of hippocampal tissue from human autopsy samples.

In addition to the susceptibility of *APOE* ε4 carriers to AD, several lines of evidence suggest that these individuals may have relatively more activation as a compensatory response to age-related changes and, perhaps, disease pathology. Older healthy *APOE* ε4 carriers have a greater cortical load of fibrillar amyloid than do noncarriers.[56] The *APOE* ε4 allele is also less efficient at inducing amyloid and cholesterol transport, which could impair regeneration of neural cells and influence synaptic plasticity.[57,58] Other studies[1,59,60] on tissue and in experimental animals suggest that neurons carrying the ε4 allele are more susceptible to the injuries and insults that accompany aging, such as oxidative damage, and are not as efficient at repair. Compatible with these findings, meta-analyses[61,62] of studies of adults recovering from trau-

matic brain injury and stroke report poorer functional outcome among carriers of the ε4 allele.

The question then arises as to why a potentially detrimental allele persists in the population. One possible explanation is that the negative effect occurs after the reproductive years. Some have postulated an antagonistic pleiotropic effect for the ε4 allele, such that it would affect cognition negatively in older age but positively in younger age.[17,63] A potential cognitive or developmental advantage of young ε4 carriers has been described in several[64-66] but not all[67] studies. Still, other purely behavioral investigations have shown a negative effect of the ε4 allele with aging but not in younger individuals.[17,20,68]

In our younger sample of participants aged 19 to 40 years we observed significantly less activation among *APOE* ε4 carriers compared with ε3 homozygotes, despite equal memory performance. The results are consistent with other studies[9,10] of younger individuals. Mondadori and colleagues[9] reported less activation in the hippocampus bilaterally across learning trials and less activity in the right hippocampus during retrieval among ε4 carriers in a sample of 34 memory-matched young people. They interpreted these findings as an expression of greater neural efficiency in the ε4 carriers, partly because in a study outside the scanner of 340 young healthy persons they found that ε4 carriers had better episodic memory than did ε3 homozygotes. This is consistent with our findings, albeit weakly significant, from a test of delayed recall performed outside the scanner in young participants in which ε2 and ε4 carriers performed significantly better than ε3 carriers. Although some studies have demonstrated better cognitive performance in young ε4 carriers as noted above, we are not aware of findings of cognitive advantage in young ε2 carriers.

Two recent studies[12,13] have observed greater BOLD response in a sample of young adult carriers of the ε4 allele. These findings, at odds with those of Mondadori et al[9] and our own work, could be related to a number of methodologic differences, including considerably smaller samples. Also, one study included, among 12 noncarriers, 4 individuals with the ε2 allele, who, as we showed, may have different activation than the ε3 homozygotes.[12] The memory task used in the other study[13] resembled more our retrieval than our encoding condition, with encoding yielding more robust findings in our study. Furthermore, when they applied the same task to older participants,[14] they obtained a pattern of activation across genotypes at odds with the largest activation studies previously published,[3,4,6,7,10] finding older ε4 carriers to have less MTL activation than ε3 homozygotes.[14] The different nature of the stimuli may also have played a role in the different results between our findings and those of the 2 recent studies in younger adults. These 2 studies used pictures of animals, one in addition to landscapes[13] and the other in addition to objects,[12] while we used scene encoding.

Scene encoding preferentially activates the posterior, rather than the anterior, portion of the hippocampal formation and the parahippocampal place area.[53,69,70] This is precisely the region where we found the greatest between-group differences in the correlation between age and activation as well as the greatest difference in activation across genotypes in younger participants. It is increasingly recognized that activation in the MTL by memory tasks has exquisite anatomic specificity.[71] Furthermore, using high-resolution fMRI, Suthana et al[72] showed *APOE* genotype-specific differences in activation in some subregions of the hippocampus, but not in others, and emphasized that the differences across genotypes may run in opposite directions in various subregions. It is interesting that our results point to the posterior hippocampus-parahippocampus as the region with the greatest differences across genotypes in the effect of age on activation. This is the same region where Suthana et al[72] speculated that older ε4 carriers would have greater activation compared with noncarriers.

Our study has some limitations. We did not include ε4 homozygotes because they are rare in the white population (approximately 3%),[73] thus precluding an assessment of the effect of 2 copies of the ε4 allele on brain activation. This potential effect should be the object of further study. We did not measure MTL volume across the age span in our sample; however, the fact that ε4 carriers have been documented to have smaller hippocampal volumes in older age than either ε2 carriers or ε3 homozygotes[51,52] argues against the direction of our findings being attributable to differences in hippocampal volume. Additionally, we did not explore genotype effects on areas deactivated by the task, which would be an important avenue of future research.

In conclusion, in a large sample spanning adult age, we found that *APOE* genotype affects MTL activation during an fMRI episodic memory task in an age- and genotype-dependent manner. Young ε4 carriers activated the MTL to a lesser extent and activation remained relatively stable with advancing age, while activation significantly declined with age among ε3 homozygotes and ε2 heterozygotes, consistent with studies of normal aging. The stepwise decrease in BOLD activation with age among these allelic variants mirrors AD susceptibility, with the greatest age-related decline among ε2 carriers and the least for ε4 carriers. The lack of decline in hippocampal recruitment with age among ε4 carriers may represent an inability to adapt to age-related changes in the brain or a compensatory response to deteriorating neural mechanisms. Based on our observation that less MTL activation occurs among younger ε4 carriers and remains relatively stable across the life-span, we cannot rule out the possibility that deficiencies in hippocampal activation in individuals at risk for AD begin at an early age. However, we also found evidence of cognitive advantage in our younger carriers. This finding is consistent with several studies showing cognitive advantage in younger ε4 carriers and, in one study, cognitive advantage in combination with less hippocampal activation. An antagonistic pleiotropic role for the ε4 allele has been suggested, and the present findings could also be interpreted as supporting this idea.

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