

Accelerated long-term forgetting in asymptomatic APOE4 carriers

In *The Lancet Neurology*, Weston and colleagues report that presymptomatic individuals carrying familial Alzheimer's disease gene mutations demonstrate accelerated long-term forgetting (ALF) over an extended 1 week retention interval compared with gene-negative controls.¹ These findings raise the question of whether ALF is also detectable in presymptomatic individuals at genetic risk of the much more common sporadic form of Alzheimer's disease.

The principal genetic risk factor for sporadic Alzheimer's disease is apolipoprotein E4 (APOE4). In white people, there is a fifteen-fold increase in Alzheimer's disease risk amongst homozygotes (E4/E4) and a three-fold increase amongst heterozygotes (E3/E4) compared with E4 non-carriers.² Standard memory tests show longitudinal differentiation according to APOE genotype at around 60 years of age,³ but evidence from cross-sectional studies of pre-symptomatic individuals at this age or earlier is inconclusive.⁴

We recruited 60 healthy white English-fluent participants (E3/E3: n=20; E3/E4: n=20; E4/E4: n=20) between 40 and 60 years of age (appendix). There were no group differences in age, sex, education, dementia family history, subjective memory ratings, or performance on a widely-used cognitive screening instrument (Addenbrooke's Cognitive Examination III; appendix). Following a protocol similar to that of Weston and colleagues, our participants learned 15 words to an 80% accuracy criterion. Rate of acquisition was calculated as gained access to words on each learning trial. Free recall was tested at 40 s, 30 min, and 1 week after the last learning trial. A 'yes/no' recognition test for intermixed target and foil words was also administered at 1 week immediately following free recall to gauge whether ALF reflects a deficit of memory storage (impaired recall and recognition) or retrieval (impaired recall but intact recognition).

APOE4 status had no discernable effect on memory encoding (figure A,B) or forgetting over the first 30 minutes (figure C). However, APOE status was associated with long-term forgetting over one week ($p=0.04$), which increased linearly with APOE4 dose ($p=0.02$; figure D). In APOE4 non-carriers, rate of acquisition correlated with long-term forgetting ($R=0.60$, $p=0.005$). This relationship was reduced in heterozygotes ($R=0.52$, $p=0.02$) and broken in APOE4 homozygotes

1 (R=0.32, p=0.17; group effect: p=0.006). Recognition memory after 1 week was also affected by
2 APOE4 carriage (p=0.01), again with a linear gene dose effect (p=0.004; figure E). Further
3 results are available in the appendix. Taken together, these findings suggest that APOE4 confers
4 a specific and dose-dependent impairment of long-term memory storage rather than of memory
5 encoding or retrieval.

6 Long-term forgetting was not associated with age or subjective memory ratings in any group,
7 and there were no interactions between genotype and age or subjective memory rating. This may
8 be partly due to the cross-sectional nature of the data and because the mean age of our sample
9 (51 years) was well below the mean age of symptom onset in APOE4 homozygotes (68 years)
10 and heterozygotes (76 years).⁵ Future studies should examine the longitudinal development of
11 ALF in APOE4 carriers as well as its relationship to amyloid beta accumulation, a relationship
12 recently identified in a familial Alzheimer's disease mouse model.⁶

13 In conclusion, ALF is present in individuals at risk not only for familial but also sporadic
14 Alzheimer's disease. We encourage further investigation into the potential utility of ALF for the
15 early diagnosis and monitoring of Alzheimer's disease, especially in clinical trials targeting pre-
16 symptomatic pathology.

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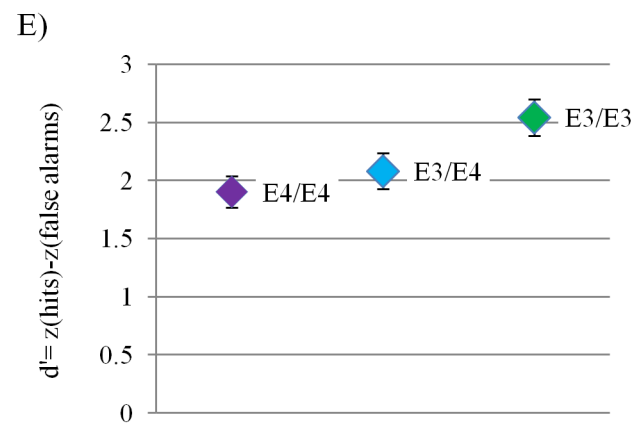
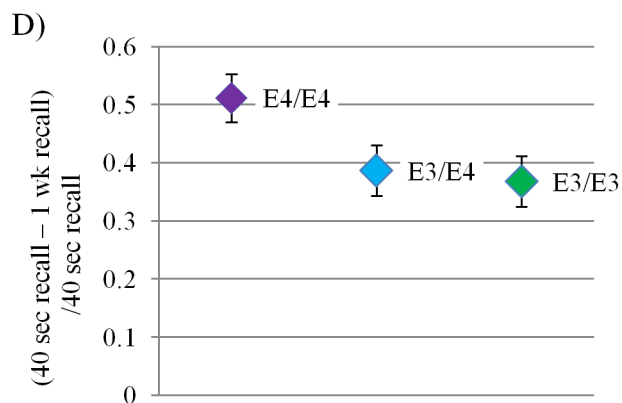
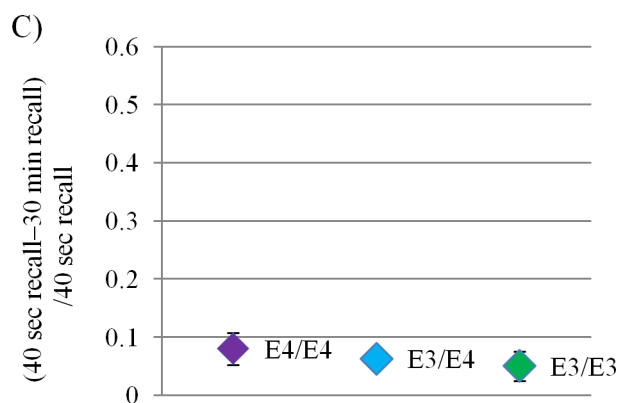
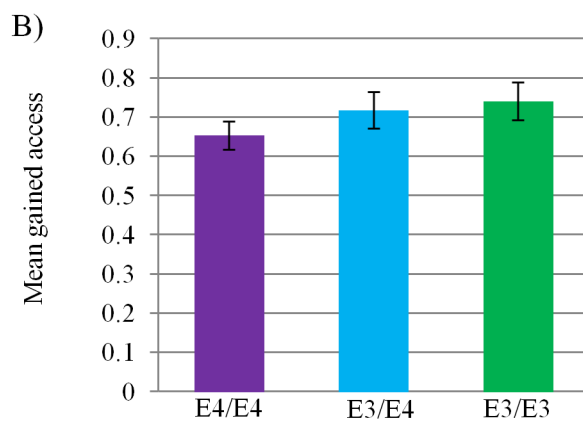
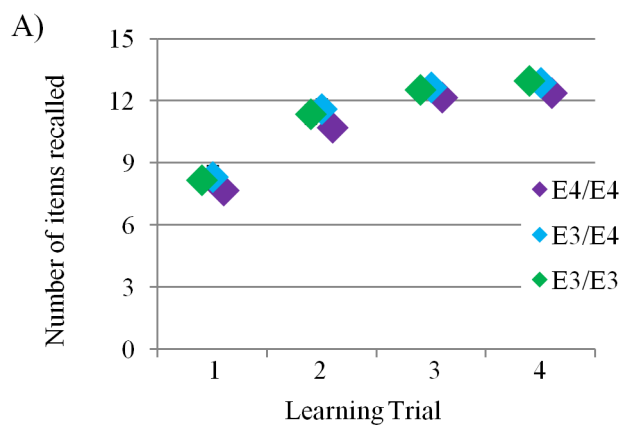
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Figure: Word list learning, standard-delay forgetting, and long-term forgetting across APOE4 gene-dose groups

Error bars represent standard error of the mean. A) Word recall of consecutive learning trials. Trial \times Group: $F(6,171)=0.18$, $p=0.98$. There were no group differences in the number of learning trials required to reach the 80% criterion of 12/15 words, $H(2)=1.35$, $p=0.51$, or in the sum learning score across learning trials 1–4, $F(2,57)=1.14$, $p=0.33$. B) Mean gained access across learning trials. No group differences were observed in mean gained access, $F(3,56)=1.69$, $p=0.18$, or in gained access over learning trials, Trial \times Group: $F(4,102)=0.85$, $p=0.50$. C) Standard delay forgetting. There were no differences in forgetting over 30 min, $H(2)=0.85$, $p=0.65$. D) Long-term forgetting. There was a significant group difference in long-term forgetting, $F(2,57)=3.35$, $p=0.04$. There was also a linear gene-dose effect on long-term forgetting, such that carriers of two APOE4 alleles forgot more information than carriers of a single allele, and non-carriers showed the least long-term forgetting, $F(1,59)=4.64$, $p=0.04$. E) Recognition memory at 1 week. Group differences, $F(2,57)=4.92$, $p=0.01$, also followed a linear trend with gene-dose of APOE4, $F(1,59)=9.24$, $p=0.004$.



Appendix

Apolipoprotein E genotyping

APOE genotyping was performed by the Oxford Biobank, using Applied Bio-systems TaqMan® SNP Genotyping Assay, C_3084793_20 and C_904973_10 corresponding to APOE SNPs rs429358 and rs7412, respectively, and run with ABI 7900HT Fast Real-Time PCR system. Haplotypes corresponding to APOE E3/E3, APOE E3/E4 and APOE E4/E4 were then identified. Genetic results were not divulged to participants or known to the experimenters at the time of data acquisition.

Methods and analyses

All testing material, including 15 word list and recognition memory probes (n=15) and foil words (n=15), were taken from the Rey Auditory Verbal Learning Test (RAVLT).

To assess learning, raw recall scores across trials were recorded, as well as the number of learning trials required to reach the 80% criterion of 12/15 words, and the sum learning score across learning trials 1-4 (/60 maximum) (figure A). In addition, a measure of gained access was calculated for each learning trial, starting from trial 2, to measure rate of acquisition (figure B).¹ Gained access is a fine-grained measure of learning which has been shown to be sensitive in Mild Cognitive Impairment and AD, and relates to metabolic activity in the medial temporal lobes.² It is calculated as the proportion of items that were not recalled at the previous trial, but that are recalled at the current trial (gained access = number of items gained on trial n / number of items not recalled on trial n-1). Mean gained access was calculated as the sum of gained access across trials, divided by the number of trials taken into account. To note, when all 15 words were recalled at one of the learning trials, gained access to the next trial was equal to 0, in which case this trial was not included in the mean gained access calculation.

Free recall was measured at 40 seconds, 30 minutes and one week, and recognition memory was measured at one week. The primary outcome measure of interest was the proportion of words recalled at 40 seconds that was forgotten over the subsequent one week delay ("long-term forgetting" = (40 sec recall - 1week recall)/40 sec recall) (figure D). Higher scores here indicate

accelerated long-term forgetting (ALF). In addition, a 30-minute forgetting score was calculated as the proportion of words recalled at 40 seconds that was forgotten over the subsequent 30-minute delay (“standard delay forgetting” = $(40 \text{ sec recall} - 30 \text{ min recall})/40 \text{ sec recall}$) (figure C). Recognition memory after one week was assessed as the difference between the z-transformed number of true positives and false positive errors ($d' = z(\text{true-positive rate}) - z(\text{false-positive rate})$) (figure E).

A total of 61 participants were tested, and one was excluded due to low ACE-III score. All 60 participants included at learning and at 30 minutes were also included in the one week follow up. One participant in the E3/E4 group was assessed at eight days rather than seven days due to inaccessibility. Visual inspection of the data confirmed that performance of this individual was not outlying from the group at any delay interval.

Scores on learning measures (number of trials to criterion, sum learning score, and mean gained access), standard delay forgetting and long-term forgetting were compared between groups using between-subjects ANOVAs. Rate of acquisition, including gained access and raw learning scores, was compared between groups using a mixed ANOVA, with Trial (gained access: 1-2, 2-3, 3-4, raw learning scores: 1, 2, 3, 4) as the repeated measure and Group as the fixed factor. Shapiro-Wilk tests of normality were conducted to test assumptions of normality. If residuals in one or more groups departed from normality for any analysis, Kruskal-Wallis non-parametric tests were used to replace ANOVAs. Planned linear trend analyses were performed where a group effect was detected, in order to assess gene-dose effects.

To assess whether the relationship between initial acquisition and long-term forgetting changed across groups, a linear regression was set up with Mean gained access and Group as regressors, and long-term forgetting as the dependent variable. The interaction of Mean gained access and Group reflected changes in the relationship between mean gained access and long-term forgetting across groups. To calculate the interaction term, the continuous variable (Mean gained access) was first centered to avoid problems with multicollinearity.³ The interaction term was then calculated using the newly centered variable and the categorical variable Group.

Similarly, linear regressions were used to examine the relationship between long-term forgetting and centered age across groups, and between long-term forgetting and subjective memory ratings

across groups. Subjective memory ratings were collected using the Everyday Memory Questionnaire (EMQ), consisting of 18 questions regarding frequency of memory problems and forgetting in everyday situations.⁴ Responses were given on a six-point scale ranging from 0 (“not at all”) to 5 (“more than once a day”), and the total EMQ score was calculated from 90 points.

Table 1. *Demographic characteristics of final sample, by group. Group means are shown where applicable, with 95% confidence intervals in parentheses. No differences were found between groups on any demographic variable.*

*Note: Family history was assessed based on the number of parents/grandparents (0, 1 or both) reported to have had clinical memory problems. *Total family history was calculated by summing number of parents/grandparents reported to have had clinical memory problems, with affected parents given a weighting of 2, and affected grandparents given a weighting of 1 (maximum score is 8).*

Genotype group	e3/e3	e3/e4	e4/e4
<i>N</i>	20	20	20
Age	50·3 (48·09–52·51)	51·8 (49·59–54·01)	51·7 (49·49–53·91)
Total years of education	14·80 (13·45–16·15)	16·30 (14·95–17·65)	16·25 (14·90–17·60)
Gender (Male/Female)	13/7	14/6	11/9
Parental History (0, 1 or 2 parents) (/2)	0·25 (0·02–0·48)	0·35 (0·21–0·58)	0·35 (0·21–0·58)
Paternal Grandparent History (0, 1, or 2 grandparents) (/2)	0·10 (-0·07–0·27)	0·21 (0·03–0·39)	0·21 (0·03–0·39)
Maternal Grandparent History (0, 1, or 2 grandparents) (/2)	0·15 (-0·04–0·34)	0·25 (0·06–0·44)	0·32 (0·12–0·51)
Total family history* (/8)	0·75 (0·20–1·30)	1·21 (0·65–1·77)	1·26 (0·70–1·83)
ACE-III Score (/100)	94·55 (92·81–96·29)	95·05 (93·31–96·79)	94·20 (92·46–95·94)

Table 2. *RAVLT word list learning, recall at 30 minutes, and recall and recognition memory at one week, by group. Group means are shown, with 95% confidence intervals in parentheses, and associated two-tailed p-values.*

	Genotype group	e3/e3	e3/e4	e4/e4	p-value
Learning	Number of words recalled across learning trials (/15 per trial)	Trial 1: 8·10 (7·26–8·95) Trial 2: 11·34 (10·44–12·24) Trial 3: 12·50 (11·77–13·23) Trial 4: 12·93 (12·16–13·71)	Trial 1: 8·32 (7·48–9·16) Trial 2: 11·60 (10·71–12·50) Trial 3: 12·65 (11·93–13·38) Trial 4: 12·87 (12·08–13·63)	Trial 1: 7·68 (6·84–8·52) Trial 2: 10·71 (9·81–11·60) Trial 3: 12·15 (11·43–12·88) Trial 4: 12·36 (11·59–13·13)	0·98
	Learning trials to criterion	3 (2·09–3·91)	3 (2·09–3·91)	3 (2·50–3·50)	0·51
	Sum learning score (/60)	44·95 (42·31–47·59)	45·45 (42·31–47·59)	42·80 (40·61–44·99)	0·33
	Gained access across learning trials (%)	Trial 1-2: 65·30 (55·01–75·58) Trial 2-3: 73·31 (61·27–85·34) Trial 3-4: 73·75 (59·27–88·24)	Trial 1-2: 63·26 (52·97–73·54) Trial 2-3: 64·68 (52·65–76·71) Trial 3-4: 71·28 (56·79–85·76)	Trial 1-2: 54·76 (45·27–64·24) Trial 2-3: 72·04 (60·94–83·13) Trial 3-4: 67·42 (54·07–80·77)	0·50
	Mean gained access (%)	74·01 (65·30–82·60)	71·76 (61·50–79·10)	65·28 (58·00–75·70)	0·18
Memory after 30 minutes	30 min recall (%)	12·00 (11·08–12·92)	11·65 (10·73–12·57)	11·00 (10·08–11·92)	0·31
	Standard delay forgetting (40s recall–30m recall) /40s recall	5·02 (-0·23–10·27)	6·30 (3·40–9·20)	8·00 (2·24–13·76)	0·65
Memory after 1 week	1 week recall (%)	8·00 (6·65–9·35)	8·15 (6·86–9·44)	6·15 (4·83–7·47)	0·05
	Long-term forgetting (40s recall–1wk recall) /40s recall	36·78 (37·06–47·31)	38·63 (29·53–47·73)	51·15 (42·53–59·78)	0·04
	1 week recognition memory (d')	2·54 (2·22–2·87)	2·08 (1·80–2·40)	1·90 (1·62–2·19)	0·01

Supplementary References

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