Reviewer's report

Title: Reduced hippocampal activation during episodic encoding in middle-aged individuals at genetic risk for Alzheimer's Disease: a cross-sectional study

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Reviewer: Mark W Bondi

Reviewer’s report:

This is a well-conceived, well-executed, and well-written manuscript that promises to contribute important findings to the neurobiological substrates of middle-aged adults at increased risk for the development of Alzheimer's disease (AD). Since little is known concerning the brain activation patterns in at-risk groups on susceptible cognitive functions prior to dementia onset (cf. Bookheimer et al., 2000, 2002; Bondi et al., 2005; Smith et al., 1999, 2002), the underlying rationale and importance of these studies is well justified. The methods of characterizing the subject samples appear sound. Furthermore, some additional rigor relating to tissue segmentation and region of interest analyses represent important methodologic improvements over many of the prior studies in this area. Nevertheless, several issues if addressed would significantly strengthen the manuscript.

The authors rightly point out that the few published studies do not demonstrate consistent findings (i.e., increased vs. decreased MTL brain response to encoding in e4 groups). However, their presentation of the literature within the Introduction is somewhat misleading in that regard. Some of the statements appear too strong: (a) that amnestic MCI is a prodromal form of AD, (b) greater hippocampal volume reductions are evident in cognitively normal e4 carriers, (c) less work [fMRI studies with e4 and non-e4 carriers] has been done with episodic memory. Each of these statements seems overly phrased. Patients with MCI (even amnestic type) only convert to AD at a rate of about 15% per year (Petersen et al., 2005). Differential hippocampal volume reductions among e4 carriers are far from a consistent finding in the literature (see Jernigan et al., 2001). With the exception of the Smith et al. studies, the fMRI work among nondemented e4 carriers has focused on episodic memory. Some more judicious treatment of the oft-conflicting literature within the Introduction would help balance the manuscript.

In the Methods, the authors state that their use of middle-aged subjects was well outside the range of possible AD onset (i.e., 15-20 years younger than the age at which AD symptoms typically develop [p. 14]), although they do not state what the average ages of onset of the parents were for their subjects. It is certainly conceivable that their subjects positive family histories included those first-degree relatives with an early age of onset. Reporting age of onset of the family member would be helpful. Also, this reviewer was uncertain if the 6 subjects excluded from the overall statistical analyses were other than the final 40 subjects, or were they from the overall sample of 40 subjects (i.e., final n = 34)? What were the APOE genotypes of the excluded subjects? Also, they do not state the handedness of subjects.

Regarding the Results section, the authors reported that the e4 group took longer to complete Trails B. This finding is consistent with Chen et al.'s 2001 (Arch Neurol) finding of the sensitivity of Trails B to conversion to AD. This reviewer was uncertain what the authors did with the Left and Right MTLs for the imaging analyses? Did they collapse Left and Right MTLs or analyze them separately? Table 2 presents findings from the Left and Right hippocampi, but again this reviewer is confused about the presentation of these findings. The first row in Table 2 presents an F-test, and the second and third rows present genotype differences. Also, Figure 2 presents a Graph of signal change in the MTL, but then the figure itself says Hippocampus. Which is it? What side is it (left, right, or both)?
Please clarify.

This reviewer would have appreciated at least a cursory inspection of the whole-brain maps. This would have accomplished a couple of complementary goals: (a) examination of differential brain response between e4 and e3 groups across multiple brain regions, and (b) comparison of the pattern and extent of brain response between studies. That is, both the Bookheimer et al. (2000) anc Bondi et al. (2005) studies presented their whole-brain analyses in addition to ROI-based analyses of the MTL. Such direct comparisons via whole-brain analysis would be helpful, especially given the differing directionality of brain response in the MTL between the current manuscript with these two prior published studies of encoding-related brain response in nondemented e4 carriers. The authors themselves discuss the possibility that e4 carriers recruit other brain regions and/or rely on other compensatory psychological processes (e.g., verbal rehearsal) during encoding to compensate for early pathological changes in MTL structure and function. [p.19]. Could this speculation not be directly assessed via whole-brain analysis albeit at reduced power from that of the ROI analyses?

Perhaps the examination of brain response by gender could be collapsed since there were significantly more women in the study and the findings did not demonstrate any significant gender by genotype interactions.

This reviewer appreciated the authors addition of voxel-based morphometric analyses of gray matter volumes within the two groups MTL ROIs. This represents an important addition to the study's rigor that the brain response is not an artifact of differing gray matter volumes or partial volume effects within the MTL region.

Perhaps the most important difficulty this reviewer had with the current manuscript is that, despite the Discussion sections nine pages, little more than one page is devoted to discussing the discrepancy with the most relevant prior encoding-related findings (Bookheimer et al./Bondi et al.) and that the authors results appear to be at odds with the compensation hypothesis. The authors cursorily discuss Bookheimer et al.'s findings and suggest that Bookheimer's activation condition primarily recruited structures associated with language rather than episodic encoding and recall. [p.16]. This is to be expected given the nature of the behavioral task that was a verbal paired-associate task. Nevertheless, significant increases in hippocampal activation were evident in the Bookheimer et al. study. Moreover, the authors fail to discuss Bookheimer et al.'s MTL ROI analyses which are directly in opposition to the authors findings. Bondi et al.'s findings with a picture-learning task also largely stand in contrast to the current manuscripts main result of decreased activation in the e4 group. However, it does appear that the current manuscripts correlations to the RAVLT are consistent with Bondi et al.'s correlations of hippocampal response and CVLT learning (i.e., positive association with the e3 group and negative or zero correlation with the e4 group). Even the studies of Smith et al. (with verbal fluency) are discrepant from one another, although admittedly they are peripheral to the focus on episodic encoding. Neurotransmitter, neurotrophic, and mitochondrial upsurges in activity in the medial temporal lobes have all been demonstrated prior to clinically evident AD, and all of which are consistent with the notion of brain compensation. In all, a better exposition of the current manuscripts reasons for discrepant findings from these prior published studies would be very helpful.

Finally, there is also the possibility that differences observed between their Encoding vs. Repeated contrast could be due to reduced activity among the e3 group during the Repeated condition, rather than increased activity during the Encoding condition. Despite the authors assertion that a low-level baseline condition could be playing into the discrepant findings across studies, it would be helpful to observe a low-level baseline contrast. Thus, examination of a differential response in the Repeated condition between the two groups relative to fixation would be helpful. If there were large group differences in the Repeated condition, then this contrast would demonstrate them. If comparability of the two groups in the Repeated condition could be assured, the authors could more confidently state that the heightened BOLD response among the e3 group in the Encoding vs. Repeated comparison
is due to the greater BOLD brain response during learning and not due to differentially reduced activity during the Repeated condition.

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests.