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

Version: 1 Date: 30 May 2005

Reviewer: Lars Nyberg

Reviewer's report:

General

Authors used fMRI to examine the effects of APOE genotype on brain activation patterns in the medial temporal lobe (MTL) during a task assumed to tax episodic memory encoding processes. The participants had at least one parent with AD and the grouping variable was carrier or non-carrier of one e4 allele. The results showed reduced MTL activity in e4-carriers. The authors conclude that this outcome provides convergent validity for the idea that the MTL exhibits functional decline associated with the APOE allele.

This is an interesting study. The authors note that only a few studies have examined brain activation differences between e4 carriers and non-carriers, and the reported results are not consistent across studies. I agree with this characterization. Therefore, additional studies are needed, and the present study has the potential to make an important contribution.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

On p. 4 it is stated that the purpose of the study was to test for reduced activation during an encoding task. During scanning, a novelty detection paradigm was used to address this question. Previously learned and novel pictures were presented in a pseudorandom order and the participants used a two-button response device to indicate learned versus novel pictures. Encoding was defined by the contrast of novel relative to familiar pictures (p. 8). I think this definition should be substantiated by reference to previous work. Buckner and colleagues have shown that encoding processes are operating also during retrieval tasks. Thus, I do not think that it is correct to characterize this as a comparison between a condition that does or does not involve episodic encoding. However, it would make sense, in light of Tulving's novelty/encoding hypothesis, to argue that 'more' encoding processes are at work in the novel than the familiar condition. Unfortunately, the authors did not include a post-scan memory test that could have addressed this issue. This limitation is briefly acknowledged (p. 21). However, I think this issue must be discussed in more detail, already in the Introduction, and references should be made to related work where a similar manipulation has been used. Also, the text should be moderated to take into account the nature of the contrast and the processes it may have isolated. For example, on p. 15 in the Discussion, the authors make reference to "a verbal encoding strategy". Is this inference valid in the light of the particular contrast that was done?

A related concern has to do with the fact that the study did not include a 'neutral' reference condition (such as a fixation cross). Therefore, the difference in signal change (as illustrated in Fig 2) is not easily interpreted. It could reflect that the MTL response to novel pictures was greater in the e3 carriers, it could reflect that the response to familiar pictures was greater for e4 carriers, or it could reflect some combination of the above alternatives. We simply do not know. Therefore, I do not think
it is correct, as for example is done in the first paragraph of the Discussion, to conclude that the e4 carriers displayed "reduced activation". Rather, I think the authors must be more careful in interpreting the direction of effect.

The strategy for data analysis was unclear to me, and in particular how many subjects were part of the 4 sub-groups after excluding 6 subjects (p. 7). I think the authors should state this more clearly. I was also unclear to me why the authors included gender as a grouping factor. Some reference is made to previous work, but it is unclear whether any previous work has documented an interaction between gender and APOE status on patterns of brain activity. Most critically, due to the subdivision, the cell sizes are very small (depending on which subjects were excluded, one cell may include fewer than 5 persons). Therefore, I find it highly questionable whether the study has sufficient power to detect a gender by APOE interaction. I would recommend the authors to consider dropping gender as a grouping variable (and delete the qualitative comparison reported on p. 12), and instead conduct a contrast between carriers and non-carriers. I suppose this contrast would be based on 15 or more subjects per group. This would make the first step with test of an omnibus F-effect redundant. Instead, the authors could conduct a small-volume correction of the t-values that result from their MTL search.

The authors correlated number of words learned on the RAVLT test with brain activity. It was unclear to me why this particular test was used. Other measures of "episodic learning ability" were also included (p. 6). Given the visual nature of the fMRI protocol, some of the others seemed more obvious candidates to me (such as Rey). If additional tests were tried but found to not correlate, this would be useful to mention (and require correction of p-values). Exactly how were the correlation analyses done--were they restricted to the MTL ROI (it was unclear whether isthmus of the cingulate cortex (p. 13) fell inside the ROI)? Finally, it seems to me that the authors should consider a test of between-group differences in the magnitude of correlations. Now it is only reported that the left anterior correlation was .58 (p<.01) for e3-carriers, but no detailed information is given for e4 carriers.

VBM was used to control for potential group difference in MTL structural volume. This was a useful addition. However, the description of the VBM analyses were quite limited. Could data from all subjects be used? I would also recommend some references to prior work on volumetric changes between e4 carriers and non-carriers.

-------------------------------------------------------------------------------

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

In fig cation 2, the y-coordinates for reported sclices could be specified.

-------------------------------------------------------------------------------

Discretionary Revisions (which the author can choose to ignore)

The way the neuropsychological data was analyzed can be better specified (t,F or what tests?). Possibly, a MANOVA could be used across tests.

The reason for constraining the analyses with the mask reported by Johnson et al (p. 10) could be better motivated. It is quite possible that genetic differences could affect brain activity outside the MTL. This is true in particular for the reversed contrast (e4 > e3).

How was the plot of signal change done (Fig 2)? Why was a 2mm radius sphere used?

Were any corrections for multiple comparisons attempted (see Table 2)?
If homozygotes were available, why were they not included?

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 outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

The only thing that applies is that we are doing related work in my lab, which perhaps relate to the last point; non-financial competing interests.