February 14, 2017

**Re: Resubmission of 55394R1**

This is a resubmission of our manuscript entitled “Generalized Psychophysiological Interaction (PPI) Analysis of Memory Related Connectivity in Individuals at Genetic Risk for Alzheimer's Disease”. After peer review, the reviewers agree that the steps required to perform a PPI analysis with functional MRI data are well-described and the study is methodologically and scientifically sound. Their main concerns were focused on the addition of specific details, clarification and adjustments to the way the findings are presented and suggestions to improve clarity in the step-by-step protocol. In the text that follows we address each comment from the reviewers. We thank the reviewers for their time and effort and believe that their comments and our subsequent revisions have results in an improved paper. We have also responded to all Editorial Comments that were not already directly addressed during the initial Editorial Review process.

**Reviewer #1:**

**Provide further details of the memory task.**

We added a note to the protocol briefly describing the task and included citations of published articles in which the task is described in detail.

**Provide information on the mean age, the gender distribution, and the MRI machine used.**

We added the mean age and the sex distribution for the participants in our study to step 1.3. We were instructed to remove the specific MRI scanner information from the manuscript during the Editorial Review process that precedes peer review. Instead, following JoVE guidelines, this information is available alongside the article in a special “Materials” table that is submitted with the manuscript. In this table we included the following information about the scanner: Siemens MAGNETOM Trio, A Tim System. We added a note to the main text referring to the Materials list after the general description of the scanner.

**Mention, before the point number 5, that the data is preprocessed at that point.**

We thank the reviewer for this suggestion. We added a note at the end of section 4 of the protocol to ensure that this is clear.

**Add additional notes; in 6.2.2, clarify what "make zero" and "centre" option means; justify the choice of the BBR registration; explain the use of (an updated) version of AlphaSim.**

We followed this reviewer’s suggestions and added notes addressing each of these points to the manuscript.

**Report the 3dFWHMx version that should be used.**

A bug in 3dClustSim was identified and fixed in the AFNI software code in May 2015. In December 2015, AFNI released an updated version of 3dFWHMx to address the issues that Eklund et al. (2016). Since these tools are used together, we have updated the manuscript to reflect that versions of both tools after released in December 2015 or later are required for valid analyses.

**Add a schematical figure of the PPI model (i.e. with the different psy\* psycho\* and PP\* conditions).**

We thank the reviewer for this suggestion and agree that a visual summary of the PPI model was missing from the manuscript. After several attempts we decided that this summary was best provided as a table with room for the title, details and notes for each EV to be provided. We have added Table 1: gPPI Model Set Up to this revision.

**Specify the direction of the group-comparison analysis in the last paragraph of the representative results section.**

We thank the reviewer for pointing out this omission. We have added a note that the representative results in Figure 3 arose from a non-carriers > carriers contrast.

**Can the authors discuss the use of the PPI approach in a data-driven analysis as opposed to a hypothesis driven approach?**

PPI seeds can be identified in a number of different ways. PPI is still hypothesis driven as it tests whether the connectivity with a region differs between conditions. Whether the seed is defined anatomically, based on the prior literature, or based on the task activity maps is independent. In the case of choosing the seed based on task activity, we are assessing whether the connectivity with regions that are activated or differentially activated by conditions or groups also has altered connectivity.

**Further discuss the correct convolution/deconvolution settings of the different regressors.**

We have added notes to the manuscript expanding on the convolution settings. Specifically, convolving the task EV by the HRF shifts the timing of the task EV to be more consistent with expected task-induced BOLD signal changes in the brain. Motion parameters do not need to be convolved by any function because they reference the realignment done at each functional volume during motion correction and thus don’t need to be adjusted by the HRF.

**Provide some rule-of-thumb limits of what can and cannot be done with PPI (i.e. subjects or signal change needed to detect changes) in terms of power limitations.**

We have not performed rigorous, iterative studies to determine rules of thumb regarding PPI analysis and statistical power. This type of analysis would be interesting to the community, but it is outside the scope of this manuscript. For a thorough discussion on power in PPI please see O’Reilly et al. (2012), which is cited in the present manuscript in the text discussing power limitations. For additional discussions on the differences in gPPI and PPI please see McLaren et al. (2012) and Cisler et al. (2013).

**Comment on the TE and why it was chosen.**

The functional scan used in the present study was designed by Dr. Mark Cohen, an experienced neuroimager and engineer at UCLA. The low TE was in part the result of optimizing against signal dropout in the medial temporal lobes, an area of great interest in this population.

**Can smoothing be problematic when carried before the time series extraction? For example, the groups used (APOE carriers/non-carriers) might differ in hippocampal volume thus differentially affecting the amount of white-matter/CSF signal introduced in the voxels of interest?**

To ensure that group differences in white matter and CSF signal introduced to the hippocampal seeds timeseries were not driving our results, we compared hippocampal seed volumes between APOEε4 carriers and non-carriers. There were no differences between groups in anterior (p=0.96) or posterior (p=0.86) seeds volumes.

**In regards to the hippocampal segmentation, Lerma-Usabiaga HBM et al., 2016 might be considered a useful alternative to writing Matlab code.**

We thank the reviewer for alerting us to this study. We have cited it in the manuscript as a potential alternative approach to writing custom code.

**Expand the section on the relevance (and the differences) of gPPI analysis vs. classic PPI.**

PPI analyses aim to investigate how different conditions or contexts alter the connectivity in the brain. Based on the work to date, there is little evidence to support the classic PPI analysis over a gPPI analysis. As shown in McLaren et al. 2012, there is no disadvantage to gPPI, but there is a high risk of false negative and false positives in the classic PPI. The difference in the two approaches is the ability to model the data. In the classic PPI, you are only modeling 2 of the conditions and are assuming that the two conditions are on the opposite side of baseline (if you have a baseline condition). gPPI allows you to accurately model all conditions and does not make any assumptions about how the conditions relate to the baseline condition. We have added a couple sentences to the discussion expanding on this topic.

**Reviewer #2:**

**Use right hemisphere anterior and posterior hippocampus as seeds as well as left hemisphere.**

We thank the reviewer for this suggestion and agree that it is not necessarily true that the right hippocampus is not involved in a language-based memory task. The scope of this manuscript, however, allows for only the presentation of a few “representative” results. We have already had to select carefully what we will include. Adding additional seed regions, while we agree would not be too complicated for an original research article, is not in keeping with JoVE guidelines and we would not be able to present those results. To this reviewer’s point, we added a note in the protocol indicating that right anterior and posterior hippocampus would be interesting seed regions for further analysis.

**Add a better description of the rationale behind the selection of the seed(s).**

We added a rationale for examining the anterior and posterior hippocampus separately as well as supporting citations. There is evidence of functional specialization along the longitudinal axis of the hippocampus. Anterior regions are input regions and associated with encoding, while posterior hippocampus is an output region associated with memory retrieval and consolidation. Thus, using these regions allows assessment of functional involvement of anterior versus posterior hippocampus in encoding vs retrieval phases of the memory task. Given the focus of this manuscript on implementation of the PPI, we have kept the background and discussion of hippocampus and memory brief.

**Justify the use of different software packages for different steps in the analysis.**

We believe that the best available tools should be used at every step of analysis and consideration of software package should be limited only to concerns of compatibility. The benefits of using a permutation-based cluster extent thresholding method include allowing for hypotheses about extent as well as height to be considered. In FSL, the threshold is set without considering data smoothness and cluster size, and therefore users often apply the default settings without reference to their specific data. 3dClustSim is related to the family-wise error method standard in the FSL software in that it takes into account the spatial correlation in the data, unlike the false discovery rate. This approach has been used in many published studies, including those that proposed and implemented the gPPI approach (McLaren et al., 2012; McLaren et al., 2014). We have not examined the results of our PPI analyses using the FSL standard thresholding approach. This analysis would be exploratory as it was not part of our original data processing stream.

**Figure 2 is difficult to follow and and does not show the main finding. Report these data in a Table.**

JoVE requests that the figures represent possible outcomes from the protocol so that readers should know what to expect and look for in their own results. We have chosen to include the within-group results in Figure 2 even though they are busy for this reason, so that readers can see what they may expect to see at this stage in their own data. Reporting these clusters in a table would be less helpful in familiarizing readers with how voxelwise PPI results might look. We feel it is especially important to keep Figure 2 in its current form since we show the between group results in Figure 3 in a so-called ‘glass brain’.

**Report or refer to published regional effects (i.e. not the connectivity) within the manuscript. It usually helps reporting them as they might help the interpretation of the data.**

We have added a brief description of these maps in *Representative Results* and a reference to where more information can be found.

**Please report more details about the MRI scanner in the methods section.**

We were instructed to remove this information from the manuscript during the Editorial Review process that precedes peer review. Instead, following JoVE guidelines, this information is available alongside the article in a special “Materials” table that is submitted with the manuscript. In this table we included the following information about the scanner: Siemens MAGNETOM Trio, A Tim System. We added a note to the main text referring to the Materials list after the general description of the scanner.

**Add a brief discussion of the results.**

JoVE manuscript preparation guidelines indicate that the Discussion should be focused on the method and protocol, which is why we did not include a thorough discussion of the results. However, if the reviewer is interested, a thorough discussion of these results can be found in the original article in which they first appeared: Harrison et al. (2016) Human Brain Mapping 37:366-380

**Add to the *Introduction* by including studies that have reported PPI connectivity effects related to individual differences in memory and emotional functions.**

We have added citations to studies of memory and emotion regulation to the *Introduction* for more thorough coverage of published applications of the PPI approach.

**Editorial comments:**

Editorial comments are italicized in bullet points below. Our responses follow each point.

*• Please note that protocol sections 1 (participant screening process) and 5 (algorithms/coding) cannot be filmed. If sections 2 is to be filmed, please provide step wise detail on how to draw blood, isolate genomic DNA, perform SNP, etc. Please note that steps that refer to manufacturer’s protocol or cite a reference on how to perform a particular method cannot be filmed.*

We note that sections 1, 2 and 5 will not be filmed.

*• Steps 3.1.1.1-3.1.1.4: Please add step wise detail on what is clicked on in the MRI software to set the parameters and acquire functional images.*

Every scanner system is different and a different order of operations would be used to achieve the same result. Also, a sequence must be designed and loaded onto a scanner before it can be run on individual subjects. This is a separate process that is outside the scope of this manuscript.

*• Step 3.1.12: How are the unrelated words, associative memory task triggered? Please mention how to exclude the first to volumes.*

MacStim is the specific program we used to administer the task. This program allows the user to trigger the task to the scanner and start on a specific TR. Since we are not able to mention specific commercial tools and software we did not include this information in the manuscript.

*• 4.1: Please add details on how to preprocess the functional data. What is done here?*

This is covered under this point in steps 4.1.#. We have a note to the manuscript indicating this.

*• 4.1.1: Please add details on how to remove the head motion artifact from the data. What is clicked on in the MCFLIRT tool to achieve this.*

MCFLIRT, like many image processing tools, is a command line program. Therefore, nothing is “clicked on” but rather the user types the command into the command line based on the instructions associated with the tool. It is not practical to include verbatim commands in JoVE articles, but we can film the user running the program from the command line as easily as we can film the user clicking on a button in a GUI.

*• 4.1.2: Please add details on how to remove non-brain tissue from the images using BET. What is clicked on in the software to achieve this.*

As with MCFLIRT above, BET is a command line tool. The user types the command into the terminal window according to the instructions provided with the program.

*• 4.1.3: Please add details on how to use the FSL motion outliers tool to identify volumes in the functional data. How are the volumes flagged? How is the output of the program used to downweight the volumes?*

FSL Motion Outliers is a command line tool. The output of this tool is a file a confound file (which the user is required to name when submitting the command so it does not have a standardized name). This file is a matrix with 1s indicating volumes that need to be downweighted. This matrix is added using the FSL FEAT GUI during processing (see step 4.2.4).

*• 4.2: Please add details on how to set up the preprocessing and GLM using GUI. What is clicked on in the software to set up the model? Please add details on what is repeated. Please provide a reference for writing a script to run the preprocessing.*

We have added more details to Step 4.2. The details of whatt is clicked on is detailed in the substeps of this step (4.2.#).

*• 4.2.1: How are the motion-corrected and brain-extracted files selected as inputs?*

This is one data file, specifically the imaging data file that has already been processed through the steps preceding this one. To select it as an input the user will select it from its location on their computer or server using a typical user interface for file navigation on a computer. To launch that window, click on “Add 4D data”. We have added this step to the manuscript.

*• 4.2.3.1: How are the 6 motion parameters added? Manually? Please mention the 6 motion parameters used in your studies.*

The motion parameters are added by selecting the files from their location on the user’s computer or server using a typical user interface for file navigation on a computer. They are text files. They are mentioned in Step 4.2.3. The output of MCFLIRT, the motion correction program, is a file with 6 columns and a row for each TR in the image (named by the user). The motion regressors are each one column of this output. We added some text to Step 4.2.3 to help make this more clear.

*• 4.2.5: Please add details on how to create the task timing text files? What is clicked on in the software? How are these files added as EVs in GLM? Please add details on how to include one for the encoding and retrieval phase. What is done here on the software? Where can one find the double-gamma HRF option? Data tab/stats tab/pre-stats tab?*

The task timing files are created using a text editor based on the design of the task. They are saved as text files. They are added as EVs in the GLM in the Stats tab under “Full Model Set Up”. This is also where the convolution options can be found.

*•* [*4.2.6.1/4.2.6.3*](http://4.2.6.1/4.2.6.3)*: Please add details on what is clicked on in the software to choose the 6/12 degrees of freedom and linear transformation.*

In the registration tab there is a drop down box where “Full Search” should be left as the default. A second drop down box where the user chooses the 6/12 DOF or BBR options. We have added this to the protocol.

*• 5.3: This step can be filmed only if performed using GUI. Please add details on how to create the anterior and posterior mask images if using GUI. Please add details on how to register. Alternatively, please remove the highlight and provide a reference.*

We have removed the highlighting and provided useful references in the Note following this step.

*• 5.4: This step cannot be filmed if it is performed using codes or algorithms.*

We have removed the highlighting from this section and others that involve command line processes.

*• 6.1.1: What is selected to exclude temporal filtering or spatial smoothing?*

The user should unclick the boxes for these options in FSL FEAT. We have added text to this step to make it more clear.

*• 6.2.1: Please add details on how to include an EV for physiological timecourse from the seed? Is the text file selected from a file location?*

This file is created by the user in Step 5.4. It will be named whatever the user names the output when running fslmeants and it will be saved where the user specifies on their computer or server. The user should save it to a location where they can access it while running FSL FEAT.

*• 6.2.2: Please mention the step numbers that describe the seed time course and one “task EV”. Please mention how to run separate models for each seed region.*

The seed timecourse is extracted in Step 5.4. The task EVs are created in Step 4.2.5. Multiple models are run by performing the steps in the manuscript and then repeating them with another seed region (since only one is included in a PPI model) if needed.

*• 7.2: What is clicked on in the AFNI software to set the voxel-level threshold?*

In Step 7.3.3, a box appears asking for the voxel-level threshold and the user types in the desired thresholding level. Step 7.2 and substeps refer to the process/programs used to determine the thresholds to use. We have changed the wording of this step to reflect that.

*• 7.2.1 an 7.2.2: These steps cannot be filmed if performed using commands/coding. Please remove the highlighting.*

We have removed the highlighting from these sections and others that involve command line processes (MCFLIRT, BET, fslmeants etc). Much of the processes are completed at the command line and it would be better if the video could include these steps (e.g., how to access the “help” or instructions for each command and then type and run the correct command).

*• 7.3.1: Please add details on how to add the parameter estimate images. How are the images chosen? What is clicked on in the SPM8 software to run the comparison?*

We have added more detail to Step 7.3.1 to make these points more clear.

*• 7.3.2: Where does the “estimate” option appear? Does is appear after the comparison is completed? What is clicked on in the SPM8 software to run the estimation process?*

Estimate is a button in the SPM8 GUI. It is always there but can only be used once an SPM.mat file has been created in Step 7.3.1

*• 7.3.3: What is clicked on in the SPM8 software to run the group comparisons? Is the voxel-level threshold set manually?*

We have updated 7.3.3 with more detail of how to run the group comparisons.

*• After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. Please highlight (in yellow) 2.75 pages or less of text (which includes headings and spaces) to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.*

We have followed these instructions.